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Since the egg changes in volume on change in concentration of the electrolytes, as well as some non-electrolytes, in the surrounding medium, it must be less permeable to electrolytes than to water, so by electrolysis one should be able to determine whether it is equally permeable to anions and kations. If an electric current be passed through the egg it begins to disintegrate first at the anode. Loeb and Budgett[1] found that the anodal disintegration of infusoria resembled the dissolution that occurs in alkali more than the coagulation in acid, and concluded that it was caused by the accumulation of kations, forming alkalis, on the outside of the animal. I found that coagulation is not the first effect of all acids. When acetic acid is passed under the cover glass of an infusorian preparation, those individuals first affected die so quickly that the process cannot be analyzed, but those specimens receiving the acid very gradually first undergo dissolution of their membranes and the protoplasm flows out, coagulating only after it reaches acid of greater concentration.

The sea urchin egg undergoes cytolysis in sea water containing acetic acid (as well as in alkalis). On passing a current through sea water in which eggs are placed the cytoplasm disintegrating at the anode is not alkaline to neutral red or to the egg pigment as it should be if the disintegration were due to alkalis massed on the outside of the egg. Furthermore, much less current is required for anodal disintegration of eggs in isotonic sugar solution than in sea water, although in the latter case more kations would be thrown against the anode end of the egg. Therefore it is concluded that the anodal disintegration of the egg is due to confined anions, and indicates relative impermeability to these anions. Probably the anions affect the protoplasm without dissociating water, and some of them may be hydroxyl ions. The anions may change the permeability of the plasma membrane and the swelling of the protoplasm occurs as an effect of the resulting diffusion of undetermined substances.

That the anodal disintegration is due to confined anions is an *a priori* conclusion if the plasma membrane be less permeable than the cytoplasm in general, to ions, since the anions migrating in the interior would be stopped by the membrane but the kations

[1] *Arch. f. d. ges. Physiol.*, lxv, 518: but see *ibid.*, cxvi, 193.

in the sea water would be free to move around the egg. Höber[1] has shown that the plasma membrane of the erythrocyte is less permeable than the interior, to ions, and this is also probably true of the egg.

In sugar solution unfertilized eggs showed anodal disintegration with less current than did fertilized eggs, indicating that the latter were more permeable to anions, or that the electrolytes had already diffused out, in either case showing an increased permeability to electrolytes.

In a molecular solution of dextrose (approximately isomotic with sea water) fertilized eggs were plasmolysed more rapidly than unfertilized eggs, indicating that the latter were less permeable to the salts exerting the internal osmotic pressure.

To sum up, the increased electric conductivity and liability to plasmolysis with molecular sugar solution and the decreased liability to anodal disintegration, of the fertilized egg, indicate that it is more permeable than the unfertilized egg, to electrolytes. How could this increase in permeability to electrolytes be related to the development of the egg?

Loeb has shown that oxygen and hydroxyl ions in the medium are necessary for development. But eggs will not develop in sea water (which contains oxygen and OH ions) unless some change is induced. Probably this change allows oxygen and hydroxyl ions to reach such a concentration *within* the egg that rapid oxidation may take place. The accumulation of carbon dioxide within the unfertilized egg lowers the concentration of hydroxyl ions. Increased permeability to electrolytes, including carbonic acid, would allow the escape of the latter and the rise in concentration of hydroxyl ions.

The increase in permeability probably causes the increased elimination of carbon dioxide and catalase, and the increased absorption of oxygen, which have been observed by Lyon, Warburg, Loeb and others.

[1] *Arch. f. d. ges. Physiol.*, CXXX, 237.

2 (527)

An inquiry into the nature of the changes in non-regenerating animals.

By **A. J. GOLDFARB.**

[*From the College of the City of New York.*]

It was shown in a recent publication,[1] that very diverse groups of animals, that ordinarily regenerate such organs as the head, tail, leg, etc., could not be prevented from so doing by destruction of that part of the nervous system supplying the amputated region and adjoining parts. And vice versa, that the lack of regeneration was not due to the absence of stimuli passing through the central nervous system.

The present studies pertain to the adult frog, selected as an example of an animal heretofore not known to replace a missing organ, such as the leg. A large series of sections of the amputated region of the leg, prepared at intervals of several days to over nine months, were studied and compared with similar sections of the leg of the newt, which regenerates its limb quite readily. Perhaps the most striking observation centers about the changes of the bone. In brief the evidence supports the view that that which retards or prevents the growth of the bony tissues correspondingly retards or prevents the growth of the adjoining tissues. It was found that while ordinarily no regeneration occurs, yet each of the tissues could, under appropriate conditions, replace the parts originally removed.

These conditions are twofold. In the first place, regeneration of the limb seems to be dependent upon the development of the bony tissues. Ordinarily the exceedingly large growth of periosteum and cartilage is succeeded by regressive changes resulting in a "callous" like structure. When this occurs all further growth changes in other tissues thereupon cease. In one particular instance, the periosteum grew so rapidly that the "anlage" of the leg and foot were distinctly differentiated before the degressive changes set in.

[1]A. J. Goldfarb, The Influence of the Nervous System in Regeneration, *Journal Exp. Zool.*, vii, 1909.

Various operations were performed with a view to preventing an early "callous" formation, and inducing a rapid growth of periosteum and thereby permitting the growth of the other tissues. Most of the legs operated in this way grew distinct "buds" or cones or various malformed or not fully developed limbs.

This incomplete or teratologic regeneration seemed to be due to the influence of a second factor, namely the rate and manner of growth of the connective tissue. This tissue is differentiated quite early, grows about and soon completely surrounds the developing embryonic tissues, apparently preventing their further growth.

Experiments are now under way, to control not only the growth of the periosteum but also this connective tissue, so as to permit, if possible, the complete regeneration of the leg.

3 (528)

Does the pituitary body compensate for thyroid insufficiency?

By **SUTHERLAND SIMPSON** and **ANDREW HUNTER.**

From the Department of Physiology and Biochemistry of the Cornell University Medical College, Ithaca, N. Y.]

Exactly one year ago we submitted to this society the results of some experiments performed on sheep with a view to throw light on the question of the vicarious relationship between the thyroid and pituitary glands. Herring and others had found histological appearances in the pituitary of thyreodectomized animals which suggested a compensatory hypertrophy in that gland, and our object was to determine whether iodine appears in the pituitary of animals from which the thyroid gland has been removed.

The operation of total thyroid extirpation was performed on ten sheep, and the pituitaries removed at death were found to contain no iodine, but we felt at the time that our results were not conclusive, and for three reasons—*first*, five of our sheep died early (six to thirty-two days) from a parasitic infection quite unassociated with the operation; *second*, the available amount of dried pituitary from the ten sheep (1.02 gram) was only sufficient

for a single analysis, and *third*, we had no sure evidence that iodine was being ingested in the food.

In order to be in a position to give a more decisive answer to the question, we have since repeated our experiments on a much larger scale. We removed the thyroid glands completely from eighteen lambs (seven to eight months old) and twelve adult sheep. After the operation they were divided into two groups and boarded out at separate farms in the vicinity of Ithaca, N. Y. The lambs (group I) were fed on clover hay, oats, beets, turnips and small quantities of cotton seed meal; the adult sheep (group II) in addition to this received daily, mixed with their food, half a gram of sodium iodide, to ensure that iodine was being ingested.[1]

No marked symptons followed the operation either in group I or group II and they were killed at the end of five to six months. The pituitary glands were removed, desiccated, and examined for iodine by Hunter's method.[2] The amount of dried pituitary obtained from each group was sufficient for two analyses (four in all) and no iodine was found.

In order to give ample time for the elimination of all circulating iodine, no sodium iodide was administered to group II for ten days before their slaughter.

Our experiments therefore prove conclusively, we believe, that no iodine appears in the pituitary of the sheep after complete removal of the thyroid gland, and assuming that the iodine containing body is the physiologically active constituent of the thyroid gland, as it is claimed to be by Reid Hunt[3] and others, it would appear that the pituitary does not compensate for thyroid insufficiency.

[1]In group I in addition to the eighteen lambs from which the thyroids were removed, one normal lamb was kept as a control. When killed along with the others, the amount of iodine found in the thyroid of this lamb (3.33 mg.) was three times as great as the average (1.11 mg.) for the eighteen at the time of thyreodectomy, five to six months previously. This showed that the natural food did contain iodine although none had been mixed with it as in the case of group II.

[2]Hunter, *Jour. Biol. Chemistry*, vii, May, 1910.

[3]Reid Hunt, Public Health and Marine Hosp. Publications, Bulletin 47, Washington.

4 (529)

The cardio-inhibitory fibers in the woodchuck (Marmotta monax).

By **SUTHERLAND SIMPSON** and **H. W. MAYES.**

[*From the Department of Physiology and Biochemistry, Medical College, Cornell University, Ithaca, N. Y.*]

In mammals the trunk of the vagus in the cervical part of its course, where it is most conveniently exposed and stimulated, is composed of afferent and efferent fibers intimately intermixed. Afferent fibers come from the pharynx, œsophagus, stomach and other abdominal viscera, larynx, trachea, bronchi and pulmonary tissue, and heart; and efferent fibers pass to the voluntary muscles of the soft palate, pharynx, larynx, to the non-striped muscle of the œsophagus, stomach and intestine, and of the trachea, bronchi and their divisions in the lungs, and to the heart.

In studying the functions of the afferent and efferent cardiac fibers by division and excitation, these cannot be separated from each other, nor from the afferent and efferent fibers belonging to other organs, except in the case of the rabbit where the depressor nerve containing the afferent cardiac fibers exists as a separate branch which can be isolated and stimulated alone.

In the woodchuck or American marmot (*Marmotta monax*) we find that the cervical part of the vagus consists of two or three distinct fasciculi which can be readily isolated in the living anæsthetised animal without injury, and stimulated individually. On ligaturing and dividing each of these strands, and stimulating the peripheral and central ends, we find that one of them alone contains cardio-inhibitory fibers. Upwards this can be followed as a distinct fasciculus to the superficial origin of the vagus nerve from the medulla oblongata, but in the lower part of the neck it appears to unite with the other bundles to form a common trunk and cannot be easily dissociated. Whether it contains afferent fibers, or efferent fibers to other organs than the heart, at the present moment we are not prepared to say, but if on further investigation we find that it consists of cardio-inhibitory fibers alone, then by tearing out the fasciculus at its point of exit from

the skull, and pursuing its course centrally by means of the method of indirect Wallerian degeneration employed by van Gehuchten, we shall be enabled to locate anatomically the cardio-inhibitory center in the medulla oblongata.

All that we feel justified in saying in the present preliminary communication, however, is that in the woodchuck one of the bundles into which the vagus nerve can be separated in the cervical region contains cardio-inhibitory fibers, while the others do not. This is unique, so far as we know, and may correspond on the efferent side to the case of the depressor nerve in the rabbit on the afferent side.

5 (530)

The relation between bile-secretion and bile-pressure.

By SUTHERLAND SIMPSON.

[*From the Department of Physiology and Biochemistry, Medical College, Cornell University, Ithaca, N. Y.*]

In the course of our work on the pressure of bile secretion in different animals, Herring and the author[1] found that in some cases the rate of bile flow was greater after the pressure was removed than it had been at the beginning of the experiment. The cystic duct having been clamped, a cannula was tied into the common bile duct and connected by means of rubber tubing to a drop recorder which marked the rate of bile flow on a slowly moving drum. A vertical glass tube mounted on a millimeter scale was introduced by means of a T-piece between the bile duct and the drop-recorder, so that by closing the exit to the drop-recorder the pressure of the secretion could be observed in terms of a column of bile.

In the course of some observations which I have since been making on bile pressure in the sheep, using the same method, I have observed on several occasions that after the bile had risen to its maximum height in the manometer, when the clamp was removed from the outflow tube the rate of flow was much greater than it had been before the pressure began to be recorded and that this increased rate of flow was maintained for a considerable time.

[1] *Proceedings of the Royal Society*, B, Vol. 79, 1907, p. 517.

One explanation of this might be that while the pressure was rising in the manometer the liver was being distended with bile and when the pressure, which was the cause of this distension, was removed the elasticity of the liver tissue led mechanically to the increased expulsion of bile. To test the correctness of this explanation, I killed one animal with an overdose of chloroform when the bile pressure had attained its maximum and at the same time released the clamp obstructing the outflow. In this case the increased rate due to the escape of the bile from the gorged liver very quickly diminished and the flow soon stopped entirely. The cause is, therefore, probably not merely mechanical.

To investigate this matter further, the escape of bile was adjusted so that a certain pressure (170 mm. bile) could be maintained in the manometer while the bile-flow was being registered by the drop-recorder, and in two cases—one sheep and one dog—it was found that the rate of bile-flow was greater than when the bile column stood at zero. At this pressure it is certain that a considerable quantity of bile was passing backwards and being adsorbed into the lymphatics so that the flow through the common bile duct did not account for all the bile that was being secreted. It is right to mention, however, that I observed this in only two experiments out of about twenty.

The most obvious explanation of this phenomenon which suggests itself to me is that the passage of bile through the lymphatics into the blood stimulates the liver cells to increased activity so that more bile is actually secreted. This is all the more probable since we know that the intravenous injection of a small quantity of bile or bile salts acts as a powerful cholagogue. In jaundice by obstruction it would appear therefore, that in the initial stages at all events, a kind of vicious circle is established whereby the bile absorbed stimulates the hepatic cells to produce more bile. This increased activity, which has for its object, no doubt, the removal of some obstruction in the bile passages, will at some stage be arrested, and may give place to partial or complete loss of function.

6 (531)

The role of the concentration of hydroxylions in the antagonistic action of K and Ca upon Na.

By JACQUES LOEB.

[*From the Rockefeller Institute for Medical Research, New York.*]

It has been proved during the last years that quite generally in animals, plants and bacteria, a poisonous solution of NaCl can be rendered harmless through the additon of small quantities of $CaCl_2$+KCl. A number of years ago I published results of experiments which indicated that the rôle of Ca in these experiments was fundamentally different from that of K. When fertilized eggs of *Fundulus* were put into *m*/2 solution of NaCl which killed the eggs in a comparatively short time it was found that small quantities of $CaCl_2$ or certain other salts with a bivalent metal rendered the solution comparatively harmless; while the same cannot be accomplished through the addition of KCl, LiCl or NH_4Cl to the *m*/2 solutions of NaCl.

Recently I was able to show this difference in the action of Ca and K in a rather striking way. The fertilized eggs of *Strongylocentrotus* were put in the following four solutions: first NaCl; second, Na+K; third, Na+Ca; fourth, Na+Ca+K. The experiments were made with neutral as well as with acid and alkaline solutions. It was found that in the neutral and acid solution the antagonistic effect of K prevailed over that of Ca, which was very slight; while in alkaline solutions the antagonistic effect of Ca was comparatively strong, while that of K was rather slight.

If newly fertilized eggs of the sea urchin are put into a mixture of NaCl+KCl, one finds that if the solution is neutral, the eggs segment rather normally and they may reach the 64 cell stage. If, however, the solution is made faintly alkaline the eggs are cytolized without segmenting.

If, however, the similar experiment is made with a mixture of Na+Ca one finds that the eggs segment better and further when the solution is faintly alkaline than when it is neutral. To give an example: In a neutral mixture of Na+Ca in one experiment only 40 per cent. of the eggs of *Arbacia* segmented and these did not go

beyond the 2 cell stage; while in the alkaline solution all segmented and reached the 8 or even 16 cell stage. It should be added that the eggs of *Arbacia* will develop in a neutral solution of Na+K+Ca to the blastula stage.

I found also that in mixtures of Na+Mg or Na+Sr or Na+Ba more eggs of *Arbacia* segmented and reached a higher stage of segmentation, when the solutions were slightly alkaline than when they were neutral. These experiments seem to indicate that for the fertilized eggs of the sea urchins the antagonistic action of Ca to NaCl makes itself felt mostly in such processes in which a concentration of HO ions higher than 10^{-7} N is required, while the K ions act antagonistically to NaCl through their participation in processes which may take place in neutral or even slightly acid mediums.

7 (532)

Digestion of protein in the stomach and intestine of the dogfish.

By **DONALD D. VAN SLYKE** and **GEORGE F. WHITE.**

[*From the Laboratories of the Rockefeller Institute for Medical Research and the Laboratory of the U. S. Fish Commission at Woods Hole.*]

Dogfish were fed by a tube with chopped, coagulated beef, and killed after 6, 12, 24, 48 and 72 hours. In the contents of the intestine and stomach determinations were made of (*a*) insoluble nitrogen, (*b*) soluble nitrogen, (*c*) soluble nitrogen in amino form,[1] and (*d*) soluble nitrogen in amino form after complete hydrolysis. The ratio $d : c$ indicates the average size of the peptides composing the peptone mixture.

During the first six hours about one half the ingested protein is dissolved and one fourth absorbed. The unabsorbed peptones are, on the average, of pentapeptid complexity. Very little transfer of stomach contents to the intestine occurs.

At the end of 12 hours 30–45 per cent. of the protein in the tract, including both solid and dissolved matter, is found in the intestine. The peptone in the stomach is broken down to the tripeptid stage, that in the intestine slightly farther.

[1]Van Slyke, these *Proceedings*.

After 24 hours 40–70 per cent. of the nitrogen has disappeared, presumably by absorption, and of that left, in both stomach and intestine, 65–85 per cent. is in solution. The peptone in both stomach and intestine is midway between the di- and tripeptid stages. It remains at this stage until absorption is complete, which occurs in 48–72 hours. After 48 hours, in two cases out of three, only 14 per cent. of the ingested nitrogen remained in the tract.

Urea is constantly present in the intestine. It is excreted with the bile, which contained, in the samples analyzed, 1.7 per cent. of urea, in which form over 70 per cent. of the total bile nitrogen is found.

8 (533)

Absorption and excretion of alimentary nitrogen.

By **DONALD D. VAN SLYKE** and **GEORGE F. WHITE.**

[*From the Laboratories of the Rockefeller Institute and the Laboratory of the U. S. Fish Commission at Woods Hole.*]

Beef and different forms of fish flesh were fed to a dog in nitrogenous equilibrium, the daily diet containing 3 grams of nitrogen, in the form of chopped meat, 65 grams of starch, and 26–27 grams of fat. The animal was catheterized 3, 6, 9, 12 and 24 hours after feeding, and the daily feces were separated by addition of lamp black to the diet. The rise in the rate of nitrogen excretion in the urine was taken as an index of the rate of absorption, the excretion of nitrogen in the feces as a measure of the completeness of absorption. The significance of the results is shown by the following figures:

Food.	Boiled Cod.	Fried Cod.	Boiled Beef.	Boiled Trutog.	Boiled Eel.	Boiled Weakfish.	Boiled Mussel.	Boiled Salt-cod.	Boiled Litorina.
Nitrogen in urine during first 9 hrs. after feeding.	1.50	1.36	1.29	1.28	1.24	1.23	1.23	1.07	1.00
Nitrogen absorbed in 24 hrs.	1.98	1.80	2.58	2.55	1.91	2.53	2.40	2.58	2.37
Nitrogen excreted in urine in 24 hours.	2.51	2.48	2.76	2.35	2.20	2.34	2.22	2.29	1.90
Nitrogen retained.	−.53	−.68	−.18	+.20	−.29	+.18	+.18	+.29	+.47

The foods in the table are arranged in rank according to the

relative rapidity with which their proteins were digested, absorbed, and metabolized, as shown by the rate of nitrogen excretion during the height of digestion (figures in top row). From the nitrogen balance (figures in the bottom row) it is evident that the order would be practically reversed if the foods were ranked according to their ability to keep the body in nitrogenous equilibrium. It is apparent that rapid digestion does not necessarily further retention of protein. Presumably there is an optimum rate of digestion, which makes possible the fullest use of its products, and this rate may be exceeded. Rapid digestion can then cause imperfect retention. "Predigestion" has recently been shown by Voit and Zisterer to have this effect.[1]

9 (534)

The cause of cardiac cohypertrophy.

By **HUGH A. STEWART.**

[*From the Laboratory of the Department of Pathology, Columbia University.*]

In a series of twenty experiments in dogs in which cardiac hypertrophy was induced by the production of aortic insufficiency, it was found that all the chambers of the heart were heavier than normal. The largest increase was in the left ventricle, 48 per cent., but there was also found a very marked increase in the auricles which, relatively to their size, was almost as great as in the left ventricle. The cohypertrophy of the auricles has also been observed in man in cases of chronic interstitial nephritis and arteriosclerosis (Hirsch).

It was found experimentally that the increased work of the left ventricle after the production of aortic insufficiency is not associated with a change in venous pressure such as has been assumed to be the direct cause of hypertrophy of the auricles.

Tracings taken of the contractions of the right auricle along with a blood pressure tracing from the right carotid artery showed that the effect of the production of aortic insufficiency is to increase the force of auricular contraction. Similarly the increased work of the left ventricle produced by the compression of the thoracic aorta will also cause increased auricular contractions without any change in venous pressure.

[1] *Z. f. Biol.*, liii, 457.

The experiments would seem to indicate that there exists a coördination between the contractions of the ventricles and the auricles of such a nature that increased systole of the former causes an increased systole of the latter without any apparent change in venous pressure.

To this condition is ascribed the cause of auricular cohypertrophy.

10 (535)

The origin of convulsions and paralysis following the intravenous injections of the hypertonic solution of sodium chloride.

By DON R. JOSEPH and S. J. MELTZER.

[*From the Department of Physiology and Pharmacology of the Rockefeller Institute for Medical Research.*]

In our experiments on the comparative toxicity of chlorides in which, among others, we studied the effects of intravenous injections of a molecular solution of sodium chloride in dogs we observed that after a certain quantity of the solution runs in, twitchings of the muscles of the entire body begin which gradually develop into more or less strong convulsions. Later these gradually grow weaker and finally subside completely, at which time also the respiration stops. The heart continues to beat for several minutes longer. This chain of events is ascribed by pharmacologists to the osmotic action of the hypertonic solution and is generally termed salt action. Loeb however demonstrated that sodium chloride exerts on the living tissue a chemical action also. In the paper embodying the above mentioned experiments we made the following two suggestions: (1) that the twitchings and convulsions are perhaps comparable to the twitching of frog muscles which develop when they are immersed in solutions of sodium chloride; the convulsive movements would be then of peripheral origin; (2) that the subsidence of the convulsions and the paralysis might be due to the curare-like action of the sodium chloride, *i. e.*, to the paralysis of the motor nerve endings.

In the present series of experiments we have tested these two hypotheses. The second hypothesis was tested by investigating

the irritability of the peripheral end of the sciatic nerve during the entire course of the experiment. It was found in every experiment that stimulation of the sciatic nerve even at the time of complete paralysis of respiration did not fail to elicit a definite motor response. This disposes of the second hypothesis; the paralysis is surely not due to a curare-like action of the sodium chloride. The first hypothesis we have tested in various ways. It is known that the twitchings of frog muscles in sodium chloride subside when calcium is added to the solution. We have therefore tried to introduce at some stage of the experiment solutions of calcium chloride into the circulation. In none of these experiments were the twitchings or the convulsions affected in any way by the addition of the calcium solution. Furthermore when the sciatic nerve was cut on one side the muscles innervated by this nerve did not take part in the twitching and convulsions. This fact was more strikingly demonstrated in experiments in which the lower half of the spinal cord was removed. In these cases the contrast between the convulsing upper half and the paralyzed lower half of the animal body was striking indeed.

It is therefore evident that the convulsions and paralysis caused by hypertonic solutions of sodium chloride have their origin neither in the muscles nor in the peripheral nerves; they originate in the spinal cord.

We may append here the brief remark that the convulsions under discussion can be greatly inhibited by intravenous injection of a non-fatal dose of potassium cyanide. We were stimulated to this latter observation by the known experiments of Loeb on the action of cyanide upon the fertilized and non-fertilized sea-urchin eggs.

11 (536)

Simultaneous graphic registration of gastric and duodenal peristalsis in rabbits; a demonstration.

By **DON R. JOSEPH** and **S. J. MELTZER.**

[*From the Department of Physiology and Pharmacology of the Rockefeller Institute for Medical Research.*]

The graphic registration of gastric or intestinal peristalsis is usually obtained from an animal with an opened abdomen while

in a saline bath and more or less under the influence of an anesthetic. This method hardly reproduces the normal condition as the act of laparotomy reduces and modifies greatly the peristalsis. In some instances the movements of the stomach have been studied from a gastric or œsophageal fistula and in the rabbit the movements can be studied graphically, as it has been discovered by Auer, in the perfectly normal and unanesthetized animal.

In the April meeting of last year we presented some tracings showing the effect of magnesium upon gastric and duodenal peristalsis. At the present time we wish to explain more fully the method we have used and to demonstrate the act of obtaining the peristaltic tracing. This rabbit was operated four days ago. The movements of the stomach and of the duodenum are transmitted to the kymograph by means of catheters which carry at one end small balloons of thin rubber. The balloon end of one catheter is introduced and secured in the pyloric part of the stomach and that of the other in the descending part of the duodenum through openings made directly in each of these parts. By means of a manometer and a rubber bulb which are interpolated between the catheter and a Marey's tambour the little balloons in the gut can be distended. When not studying the peristalsis the soft catheters are secured by means of a bandage around the animal. The peristalsis is usually obtained when the animal is in a normal crouching position. Soon after the operation there is very little peristalsis to be noticed. In the first few days the peristalsis improves from day to day in character, intensity and regularity. Three to five days after the operation the peristalsis seems to assume a normal character, which, however, we shall not discuss in the present communication. We wish to state, however, that in our earlier experiments in which the duodenal balloon also was introduced through the stomach we lost most of the rabbits within 24 or 36 hours after the operation. The administration of a small dose of physostigmin soon after the operation improved our results greatly. At present we introduce the catheter into the duodenum directly through an opening in the wall of the latter. Our results are now very satisfactory.

12 (537)

The method of inheritance of two sex-limited characters in the same animal.

By T. H. MORGAN.

[*From the Department of Zoölogy, Columbia University.*]

At the last meeting of the society I reported the occurrence of a white-eyed mutant in a pedigree culture of the fly *Drosophila ampelophila*. The mutant bred to his red-eyed sisters produced 1,237 red-eyed (male and female) and 3 white-eyed (male) individuals. This sporadic occurrence within the strain is due to further sporting in the eggs of certain females. The same strain has continued to produce white-eyed mutants and these are always of the male sex.

This white-eyed condition has shown itself to be sex limited in its inheritance, *i. e.*, in certain combinations the character is transferred to one sex only. For instance—If a white-eyed male is bred to a red-eyed individual all of the offspring are red-eyed (males and females). These inbred produce red-eyed males and females and white-eyed males. The reds are to the whites as 3 to 1. Thus half of the grandsons inherit the new character but none of the granddaughters.

This result can be explained by means of a very simple hypothesis. If R = red eyes; W = white eyes, and X = the sex factor (one X being a male and XX a female); then the red female fly will be RXRX and her eggs RX and RX; the white male will be WXW and his two classes of spermatozoa WX and W. When crossed the following combinations result.

RX – RX
WX – W
RXWX – RXW
red female red male

When these individuals are paired the outcome is shown by the following formulæ:

RX – WX
RX – W
RXRX = red female
RXWX = red female
RXW = red male
WXW = white male

Another mutant that shows itself to be sex limited in inheritance has appeared in these same cultures. A few males appeared with wings half the normal length.

One of these short-winged males bred to a normal female of another stock produced long-winged males and females. These inbred have produced 5,856 long-winged males and females, and 85 short-winged males (no short-winged females). Here again the character is transmitted from the grandfather to some of his grandsons but to none of his 3,000 granddaughters.

The next question to determine is the relation in inheritance of these two characters, both sex limited. At present I can give only a provisional statement, since the experiment is still in progress.

In the experiment last described the short-winged male had red eyes. He was paired to a long-winged white-eyed female. The offspring consisted of red-eyed females and white-eyed males (the normal result for this combination). All had long wings. These were inbred and produced in the second generation red-eyed, long-winged males and females, white-eyed long-winged males and females, white-eyed short-winged males and red-eyed short-winged males.

In other words, neither white eyes nor red eyes were limited to the short-winged males.

The results are difficult to explain fully owing to the great deficiency of the short-winged forms, due possibly to incompatibility. Let R = red eyes; W = white eyes; L = long wing; S = short wing; X = the sex factor; then: RXSWS = the red-eyed short-winged male, and WXLWXL = the long-winged white female. Their germ cells will be

WXL – WXL
RXS – WS
WXLRSX – WXLWS
red ♀ long white ♂ long

The germ cells of these individuals, and the results of their combination should be:

WXL – WXS – RXL – RXS, red ♀	
WXL – WS	white ♂
WXLWXL = white ♀	long wings
WXLWXS = " "	"
WXLRXL = red "	"

WXLRXS = red ♀ long wings
WSWXL = white ♂ " "
WSWXS = " " short "
WSRXL = red " long "
WSRXS = " " short "

NOTE ADDED NOVEMBER 20.

The converse cross, viz., short-winged, white males by long-winged, red females gives also in the second generation besides long-winged, red- or white-eyed, males and females, short-winged red- or white-eyed *males*.

Heterozygous white females (WXLWXS) by short-winged red males gives long-winged red females, short-winged red females, long-winged white males and short-winged white males.

13 (538)

The glycogenolytic strength of blood serum from the pancreatico-duodenal vein and from the femoral artery, and of lymph from the thoracic duct, as affected by stimulation of the great splanchnic nerve.

By **J. J. R. MACLEOD** and **R. G. PEARCE.**

[*From the Department of Physiology, Western Reserve University Cleveland, Ohio.*]

The distribution of diastatic ferment (glycogenase?) in the animal body would lead one to conclude that its site of production is in the pancreas. Thus:

1. Extracts of this gland possess a glycogenolytic activity which is enormously greater than that of extracts of any other gland, or of blood serum.

2. Blood serum contains the next largest amount of glycogenase.[1]

These considerations prompted us to see whether blood from the pancreatico-duodenal vein is stronger in glycogenase than blood from the femoral or carotid arteries. They were found to be the same. We have recently repeated the observations with the modification that some of the samples of blood were collected during

[1]Macleod & Pearce, *Amer. Jour. of Physiology*, 1910, xxv, p. 255; *cf.* also Wohlgemuth and Benzur, *Biochemische Zeitschrift*, 1909, xci, p. 460.

stimulation of the great splanchnic nerve. In these experiments we have also determined the glycogenase in lymph, collected from the thoracic duct. The experiments were conducted in the usual manner[1] and in most cases controls with 0.5 per cent. soluble starch were run. These gave similar results to those in which glycogen

Experiment No.	Nature and Amount of Fluid Used.	Experimental Condition.	Glycogen (Dextrose) in Incubation Flasks.			Remarks.
			At Start.	After Incubation.	Disappeared.	
III.	Fem. art.	Before stimulating gt. spl. nerve.	0.087	—	—	Incubated 3 (?) hrs. 1 c.c. serum. Starch test gave dextrines first with *.
	Do.	After 15 min. stim.	"	0.014	0.073	
	Pan. duo. vein.	Before stim.	"	0.019	0.068	
	Do.*	After 15 min. stim.	"	0.011	0.076	
	Lymph.	Before stim.	"	0.020	0.067	
	Do.	After 15 min. stim.	"	0.012	0.075	
IV.	Fem. art.	Before stimulating gt. spl. nerve.	0.185	0.077	0.108	Incubated 3 hours. 1 c.c. serum. Starch test gave dextrines in 60 min. with all.
	Do.	During 1 hour stim.	"	0.077	0.108	
	Do.	30 min. after stim. off.	"	0.082	0.103	
	Pan. duo. vein.	Before stim.	"	0.082	0.103	
	Do.	During 1 hour stim.	"	0.082	0.103	
	Do.	30 min. after stim. off.	"	0.080	0.105	
VI.	Fem. art.	Before stimulating gt. spl. nerve.	0.141	0.067	0.074	Incubated 4½ hrs. 1 c.c. serum in each case. Starch test gave dextrines first with *.
	Do.	During 30 min. stim.	"	0.040	0.101	
	Do.	45 min. after stim. off.	"	0.062	0.079	
	Pan. duo. vein.	Before stimulating.	"	0.060	0.081	
	Do.	During 30 min. stim.	"	0.059	0.082	
	Do.	45 min. after stim. off.	"	0.051	0.090	
	Lymph.	Before stim.	"	0.053	0.088	2 c.c. serum incubated 3½ hours.
	Do.*	During 30 min. stim.	"	0.026	0.115	
	Do.	45 min. after stim. off.	"	0.040	0.101	
VIII.	Fem. art.*	Before stim.	0.146	0.081	0.065	Incubated 1 hour. 1 c.c. serum. Starch test gave dextrines first with * * *.
	*	During 20 min. stim.	"	0.081	0.065	
	*	80 min. after stim. off.	"	0.070	0.076	
	Lymph.	Before stim.	"	0.095	0.051	
		During 20 min. stim.	"	0.099	0.047	
		60 min. after stim. off.	"	0.101	0.045	
IX.	Fem. art.	Before stim.	0.140	0.065	0.075	Incubated ½ hr. 5 c.c. serum.
		During 80 min. stim.	"	0.058	0.082	
		60 min. after stim. off.	"	0.047	0.093	
	Pan. duo. vein.	Before stim.	"	.—	.—	
		During 80 min. stim.	"	0.058	0.082	
		60 min. after stim. off.	"	0.060	0.080	

was employed. The incubation periods varied from 30 minutes to 4 hours and 30 minutes, and the amounts of serum or lymph varied

[1]Macleod & Pearce, *loc. cit.*

from 1 c.c. to 5 c.c. By thus varying the conditions, chances of error are greatly eliminated. Controls were run in all the experiments except in some of the estimations of experiments 3 and 8 in which there was not sufficient serum or lymph for this purpose. Table I gives the results of these experiments.

Consideration of Results.—In experiments IV and VIII there was no evidence of increase of glycogenase during stimulation of the splanchnic nerve. There was a slight increase in the ferment contained in the femoral blood after the stimulation in experiments VIII and IX, but, since this was 60–80 minutes after the stimulation had been removed, the increase cannot be due to the stimulation. In experiment III there was a slight increase both in the pancreatic vein and in the lymph immediately after the stimulation. In experiment VI there was an increase in the lymph and in the femoral artery blood during the stimulation. The only experiment, therefore, in which splanchnic stimulation certainly caused a rise in glycogenase was No. VI. The increase occurred primarily in the lymph and secondarily in the systemic blood. It fell off in both after the stimulation was removed. We are not in a position at present to determine whether this result is of any importance in the metabolism of carbohydrates in the animal body, but we do not believe that it has any relationship to the increased glycogenolysis which occurs in the liver as a result of stimulation of the splanchnic nerve. Did it have this relationship we should expect the increase in glycogenase to occur in the blood of the pancreatico-duodenal vein rather than in the lymph.

14 (539)

The influence of an inoculation with tumor material of experimentally decreased virulence upon the result of a second inoculation with tumor material of experimentally decreased virulence.

By **ELLEN P. CORSON WHITE** and **LEO LOEB.**

[*From the Laboratory of Experimental Pathology of the University of Pennsylvania and from the Department of Pathology of the Barnard Free Skin and Cancer Hospital, St. Louis.*]

Eight years ago one of us has shown that through the influence of chemical and physical factors it is possible to decrease the energy of growth of tumor-cells without killing the cells.[1] While a certain degree of injury kills the cells, a graded decrease in the action of the external agency leads to corresponding gradations in the decrease in the energy of growth of the cells. Thus it is possible to obtain tumor material which is still alive, but which gives rise to a very insignificant growth in many instances, and the initial growth of which is in many cases followed by a spontaneous retrogression. At that time we considered the possibility that such tumor material of experimentally decreased virulence might serve as a vaccine which might provide a certain protection against a second injection with more virulent material.

We tested therefore the influence of a first injection of such material upon the growth of a subsequent inoculation with tumor material. In the majority of cases both inoculations were separated by an interval of 10–20 days.

In order to obtain a more sensitive reagent indicating the presence of even a slight immunity, we used as material for the second (or sometimes third) test-injection not virulent tumor pieces, but again tumor material of experimentally (through heating) decreased energy of growth. Our results were as follows:

1. If the tumor material of experimentally decreased virulence did not give rise to tumor formation, no immunity was noticeable, even if the mice had received two or three preparatory injections

[1] *Virchow's Archiv*, Bd. 172, 1903, p. 395.

at various intervals. In a number of cases the last inoculation gave rise to even larger tumors than in the control mice which had not received any preparatory inoculation. In cases in which mice were injected with a suspension of finely divided tumor material of (through heating) diminished virulence, the same lack of immunity was observed on subsequent inoculation with tumor pieces of decreased virulence. The number of mice which we treated with a preparatory injection of fine tumor suspension is however as yet relatively small and these experiments will therefore have to be continued.

2. If the preparatory inoculation with material of decreased virulence led to a temporary tumor growth, which latter was followed by a spontaneous retrogression an immunity against a subsequent inoculation with virulent tumor material was obtained in many cases. This observation is in accordance with the findings of other investigators. This immunity is however by no means absolute and it will be necessary to determine in further experiments upon what factors such gradations in acquired resistance depend.

3. In many cases mice in which a tumor has begun to grow after the first inoculation of tumor material of decreased virulence, can be successfully inoculated a second time with material of decreased virulence, and in some experiments the tumors derived from the second transplanted piece grow as well in mice in which the first preparatory inoculation had given rise to tumor growth as in such mice in which the first pieces had not given origin to tumor growth, and in such animals two tumors are growing simultaneously; but in the majority of cases we find that only one tumor grows after two successive inoculations with tumor material of decreased virulence. If the preparatory inoculation had been followed by tumor growth, the second inoculation gave frequently a negative result and vice versa. And especially if the tumor resulting from the first inoculation is growing very actively, a second inoculation with material of decreased virulence is in the large majority of cases not followed by tumor growth. This observation would therefore point to the conclusion that in a certain number of cases the growth of a tumor protects to some extent against a subsequent inoculation with material of de-

creased virulence. But in as much as in a number of experiments such an excluding action of a first inoculation was not noticeable, we are not yet ready to state positively that such an immunizing effect of a growing tumor exists. We are however inclined to believe that a number of variable factors (especially a variable energy of growth of the first and second tumors) are present and that such factors complicate the results. In further experiments we shall endeavor to analyze such variable factors.

15 (540)

Supplementary report on attempts to immunize against tuberculosis.

By **J. P. ATKINSON** and **C. B. FITZPATRICK.**

[*From the Department of Health, City of New York.*]

We have sought to employ the resistance acquired by or embodied in the serum of the healthy animal in its life struggle against a deadly infection with tuberculosis.

The depression reaction produced by injections of tuberculous serum into animals sensitised with tuberculin, a description of which we have already published has led us to this investigation.

After employing a number of methods for utilizing the serum of healthy animals which had been infected with tuberculosis, we are duplicating one which seems to have given promising results.

Method 1—Begun December 2, 1909. The result of this experiment was reported at the meeting of the American Association for the Advancement of Science, December 27, 1909.

One rabbit was given a sensitising dose of 1 c.c. tuberculin subcutaneously and beginning two days later this rabbit received the following:

Dec. 4,	1 c.c	Tuberculous rabbit serum.
" 6,	"	" " "
" 8,	"	" " "
" 10,	2 c.c.	" " "
" 14,	"	" " "
" 16,	"	" " "
" 18,	"	" " "

A rest of ten days was given the experimental rabbit which was

then given a fatal dose of a culture of bovine tuberculosis intravenously. Two control rabbits were given an equal amount of the same culture. The controls lived 30 days during which time they lost weight and presented a typical tubercular appearance. The autopsy showed marked and characteristic lesions of tuberculosis.

The experimental rabbit lived 39 days and kept in good condition up to one week before its death and then lost weight rapidly. The autopsy showed the same marked and characteristic tubercular lesions as the controls.

The fact of the long-continued good physical condition of the experimental rabbit encouraged us to repeat this experiment with some changes.

January 8, 1910. Two rabbits were sensitised with ¼ c.c. each of crude tuberculin which was followed on the twelfth inst., by injections of 3 c.c. each of tuberculous rabbit serum. This procedure was repeated three times and the serum alone given six times.

The following is the table of injections, all subcutaneous.

January 8, each ¼ c.c. crude tuberculin.
" 12, 3 " tuberculous rabbit serum.
" 14, ¼ " crude tuberculin.
" 17, 3 " tuberculous rabbit serum.
" 19, ¼ " crude tuberculin.
" 21, 5 " tuberculous rabbit serum.

One rabbit died on January 21. The other one received on

January 24, ¼ c.c. crude tuberculin.
" 26, 5 " tuberculous rabbit serum.
" 28, 2½ " " " "
" 30, 2½ " " " "
February 3, 3 c.c. tuberculous rabbit serum.
" 5, 5 " " " "
" 7, 5 " " " "

A rest of 11 days was given the surviving rabbit, and then on February 18, 1910, it was given a fatal dose of bovine tuberculosis intravenously. Two control rabbits were given the same amount of the bovine tuberculosis culture.

One control died April 5, the second control died July 11; both these rabbits showed marked and characteristic tubercular lesions. The experimental rabbit died July 17, from pneumonia.

The autopsy showed that the lungs, liver, kidneys, suprarenal capsule, spleen and glands were free from tubercular infection.

Both controls lost weight consistently until death.

The experimental rabbit held its weight up to a week before its death when it lost weight very rapidly.

This rabbit appears to have been successfully protected by injections of tuberculin and tuberculous rabbit serum from a fatal dose of a culture of bovine tuberculosis. We are now duplicating this experiment with two experimental rabbits and three controls.

On June 25, 1910, two variations of the previous methods were used. Two rabbits were used for each experiment. Variation 1 consisted in alternate injections of crude tuberculin and tuberculous rabbit serum as follows:

June 25, each rabbit ¼ c.c. tuberculin.
" 27, " " 2 " tuberculous rabbit serum.
" 28, " " ¼ " tuberculin.
" 29, " " 2 " tuberculous rabbit serum.
" 30, " " ¼ " tuberculin.
July 1, " " 2 " tuberculous rabbit serum.
" 2, " " ¼ " tuberculin.
" 4, " " 2 " tuberculous rabbit serum.
" 5, " " ¼ " tuberculin.
" 6, " " 2 " tuberculous rabbit serum.
" 7, " " ¼ " tuberculin.
" 8, " " 2 " tuberculous rabbit serum.

Variation 2 consisted in alternate injections of tuberculous rabbit serum and that portion of the tubercle bacillus split by alcoholic potash which is insoluble in the alcohol. Vaughan claims that this portion of the tubercle bacillus sensitises but does not poison. This is true of the preparation we used as will be described further on.

The following table gives the injections:

June 25, each rabbit 1/10 gm. insoluble residue.
" 27, " " 2 c.c. tuberculous rabbit serum.
" 28, " " 1/10 gm. insoluble residue.
" 29, " " 2 c.c. tuberculous rabbit serum.
" 30, " " 1/10 gm. insoluble residue.
July 1, " " 2 c.c. tuberculous rabbit serum.
" 2, " " 1/10 gm. insoluble residue.
" 4, " " 2 c.c. tuberculous rabbit serum.
" 5, " " 1/10 gm. insoluble residue.
" 6, " " 2 c.c. tuberculous rabbit serum.
" 7, " " 1/10 gm. insoluble residue.
" 8, " " 2 c.c. tuberculous rabbit serum.

(One of these rabbits was bitten and died from a resulting infection, August 1.)

These four rabbits with three controls were each given an intravenous injection of a lethal dose of bovine tuberculosis on July 22, 1910, after an interval of 12 days since the last injection of tuberculous rabbit serum.

These rabbits died and showed marked and characteristic lesions upon autopsy except the rabbit which died on August 1, as the result of a bite and one that died August 22, and was not autopsied. This animal, however, showed physical signs of tuberculosis before death.

The following table shows the time of death of the experimental rabbits and the controls.

Controls 1—died August 8.
" 2— " " 12.
" 3— " " 15.

Experimental rabbits—Tuberculin and Tuberculous rabbit serum.
1—died August 12.
2[1]— " " 22.

Experimental rabbits—Tuberculous rabbit serum and insoluble residue.
1—died from infected bite August 1.
2— " August 10.

In order to test the properties of the products obtained from the treatment of the tubercle bacilli according to the directions of Dr. Vaughan the following tests were made.

Some of the soluble portion injected subcutaneously into a guinea pig produced the following symptoms: nervousness, chilliness, weakness in the hind quarters, convulsions and death in about 45 minutes.

Injections of the alcoholic insoluble portion did not cause symptoms of any sort.

A small quantity of this insoluble portion was injected into each of three guinea pigs. After three weeks two of these pigs were injected intraperitoneally with an emulsion of tubercle bacilli, from which the split products had been prepared, with the following results.

Pig No. 1. After 30 minutes, restlessness followed by weakness in the hind legs; violent convulsions followed by extreme fatigue. These convulsions continued during the day and the pig died finally in a convulsion 6 hours after the injection.

[1]No. 2 had lost a great deal in weight and was undoubtedly tuberculous.

The autopsy showed undissolved bodies of tubercle bacilli spread over the omentum.

Pig No. 2. Some restlessness but no other sign that it was affected by the injection. Two days later this pig was seized with a convulsion and died.

The autopsy showed undissolved bodies of tubercle bacilli not spread over the omentum but collected together in small groups. The edge of the spleen was covered with small abscesses which did not show tubercle bacilli.

The serum of rabbits treated according to experiment two (2) acquired a clumping property for the tubercle bacilli. This was shown by each of the two rabbits that are now duplicating the successful experiment No. 2.

An emulsion of bovine tuberculosis was mixed with clear serum drawn from these animals six days after the last injection.

A mixture of this serum from each rabbit and a uniform suspension of tubercle bacilli after standing overnight at room temperature was found to cause well defined clumping. The control in normal sodium chloride solution was not clumped.

A knowledge of substances which counteract the toxic effects of the infecting organism is of great value in attempts at immunization.

The fact that "tuberculin" the toxic extract of the tubercle bacillus is an arterial blood depressor immediately suggests that the extract of the suprarenal gland might antagonize it. We have carried out experiments of this nature and find that according to the amount of tuberculin and suprarenal extract in the mixture one can produce a depression or rise at will.

The depressor substance in tuberculin then can be measured by suprarenal extract of known strength.

If the depressor substance in tuberculin is a necessary constituent for tuberculin as a diagnostic and therapeutic agent, then we have an easy and simple method of measuring its strength.

We have injected tuberculin into dogs from which we have removed one and both suprarenals. The results bear out the theory, but have brought to light so much that is new and interesting that we will reserve the discussion for a future paper.

SCIENTIFIC PROCEEDINGS.

ABSTRACTS OF THE COMMUNICATIONS.

Forty-first meeting.

The Rockefeller Institute for Medical Research. December 21, 1910.

16 (541)

Retention of normal polarity in centrifuged stems of *Tubularia*.

By **MAX W. MORSE.**

[*From the Harpswell Laboratory and the Biological Laboratories of Trinity College, Hartford, Connecticut.*]

Stems of the hydroid, *Tubularia crocea*, were cut from the colony, which had been taken directly from the sea. The hydranths were removed by cutting them from the stems immediately below their attachment. A small branch from the stem was left intact to serve for orientation, and the hydranth which belonged to this branch was cut off.

These stems were placed in specially prepared tubes fitted to a hematocrit centrifuge, operated by hand. Rotation was made at varying speeds and for varying intervals of time ranging from a minimum speed of rotation of about two hundred and forty per minute to a maximum of about six hundred rotations per minute. A greater speed than this resulted in forcing the contents of the perisarc tube out at the distal end. The periods of time of rotation varied from a minimum at highest speed of one minute to a period of maximum operation at medium number of rotations per minute (approximately three hundred, the centrifuge being about one-in-four, but there was no speedometer available to accurately measure the number of rotations at the higher speeds), for one half hour.

The behavior of *Tubularia* stems under all of these conditions was uniform and unvarying. When regeneration occurred, which was true in 100 per cent. of the cases under observation, as the stems lay horizontally in finger-bowls, the hydranth appeared at

the originally *distal* end of the stem, regardless as to whether the distal or proximal end of the stem had been directed centrifugally or centripetally in the experiment and regardless of the fact, that as in the higher speeds, the contents of the perisarc tube were compressed into the end of this tube which was directed centrifugally in rotation. This compression varied with the centrifugal force involved, but at the higher speeds, the contents which filled the tube for a distance of four centimeters, would be compressed, in the experiment, into a space measuring about five millimeters. When regeneration took place, the red pigment which marks the future hydranth pole could be seen collecting in the compressed protoplasm and gradually it migrated up the tube of perisarc until it reached the end of this tube, whereupon the tentacles and other parts of the normal hydranth appeared.

That the red pigment has no rôle as a "formative stuff" has already been shown by Morgan according to evidence derived from another method of approaching the point and the present set of observations appears to show that if any stratification of "formative stuffs" occurs in the normal stem of the hydroid, whereby hydranth forming stuffs and stolon forming material are relegated to their respective ends of the stem, these stuffs are not responsive to the action of centrifugal force in the degrees used in the experiments or else they become rearranged when the centrifugal action has ceased. The generalization may be made that polarity in *Tubularia crocea* cannot be altered by the action of centrifugal force, in shifting "organbildende Bezirke" from one end of the hydroid stem to the other.

17 (542)

Creatin and creatinine metabolism during convalescence after typhoid fever.

By **NELLIS B. FOSTER.**

[*From the Wards of the New York Hospital and the Laboratory of Biological Chemistry of Columbia University at the College of Physicians and Surgeons, New York.*]

On account of the apparently intimate connection between "muscle efficiency" and the output of creatinine in human urine

it seemed of some moment to investigate the creatin and creatinine excretion during convalescence from acute febrile disease. Patients convalescent from typhoid fever were selected because during the course of this disease there is considerable loss of muscle tissue, and on that account if creatin or creatinine take part in the synthetic processes of muscle regeneration, their utilization for this purpose might be more easily detected than in conditions where the metabolism is less active. Young adults and children were used exclusively, since it is reasonable to suppose that in such subjects anabolism occurs at its height.

The diet in all cases consisted of milk, eggs and cereals exclusively.

The following tables are compiled from the records of analyses which were made during two weeks of convalescence in each of these cases.

From these tables it may be noted that when creatinine is ingested under the conditions of these experiments a large part of the material is recovered in the urine, but there is always a loss which remains to be accounted for. When creatin is fed it can not be recovered as creatin in the urine, unless the patient is on a diet very rich in protein and the amounts of administered creatin are large. A slight increase in creatinine excretion which at times follows the ingestion of creatin is too small to warrant any conclusion.

The occasional presence of creatin in the urine of these patients is not to be explained by any observation made clinically. All of the patients were without fever during the convalescence and relapses occurred in no instance. The presence of even traces of creatin in urine under these circumstances is of interest in its bearing upon the earlier observations of Munk.[1]

[1] *Deutsch. Klinik*, 1862, p. 300.

Case I. (Weight 50 K.)

Day of Observation.	Amount of Urine, c.c.	Total N, gm.	Creatinine, gm.	Creatin, gm.	Remarks. Amount Fed.[1]
1	1,820	9.12	0.910		
2	1,450	8.96	0.838		
3	1,150	8.26	0.810 (average 0.853 gm.)		
4	1,400	7.09	0.994		0.47 gm. creatinine
5	1,930	8.84	0.965		
6	1,480	9.89	0.915		(recovered 0.3 gm.)
			Case II. (Weight 28.6 K.)		
1	850	4.70	0.456		
2	1,430	9.35	0.448		
3	1,770	12.72	0.478		
4	1,490	7.99	0.440 (average 0.455 gm.)		
5	1,730	12.27	1.073		0.94 gm. creatinine
6	1,910	11.52	0.439		(recovered 0.62 gm.)
			Case III. (Weight 60 K.)		
1	1,150	10.99	0.831	0.005	
2	2,020	10.72	0.997 (average 0.914 gm.)	0.004	
3	1,100+	9.74+	0.781+	0.006	
4	1,720	11.15	1.734	none	1.639 gm. creatinine.
5	1,710	10.12	0.968	0.058	(recovered 0.82 gm.)
			Case IV. (Weight 28.1 K.)		
1	1,000	6.63	0.250	0.105	
2	1,150	6.53	0.220	0.125	
3	1,010	8.63	0.287 (average 0.252 gm.)	0.063	
4	1,000	8.09	0.310	0.074	.3 gm. creatin.
			Case V. (Weight 45.9 K.)		
1	1,305	3.27	0.741	——	
2	1,120	9.01	0.605	——	
3	1,790	4.00	0.895	0.004	
4	1,410	10.41	0.635 (average 0.719 gm.)	0.036	
5	1,105	8.34	0.500	0.046	0.66 gm. creatin.
6	1,220	9.70	0.588	0.010	
			Case VI. (Weight 59.5 K.)		
1	1,260	9.88	0.788	——	
2	1,650	12.56	0.765 (average 0.776 gm.)	——	
3	1,700	10.01	0.935	0.018	1.31 gm. creatin.
4	1,720	10.41	0.989	0.017	
5	1,430	11.29	0.667	——	
			Case VII. (Weight 40 K.)		
1	1,300	32.57	0.438	0.065	
2	580	25.23	0.451	0.109	
3	1,370	34.94	0.444 (average 0.444 gm.)	0.107	
4	1,410	32.99	0.524	1.411	3 gm. creatin.
5	840	26.46	0.616	0.338	

[1]The amounts of creatin and creatinine given were computed from the weight of substance on the basis of quantitative estimation by Folin's method.

18 (543)

Creatinine and creatin metabolism in dogs with Eck fistula.

By **NELLIS B. FOSTER** and **HENRY L. FISHER.**

[*From the Laboratory of Biological Chemistry of Columbia University at the College of Physicians and Surgeons, New York.*]

In connection with certain studies of hepatic function the creatin and creatinine metabolism were investigated in two dogs with Eck fistula. This subject was studied last year by London and Boljarski.[1] They found that the administration of creatinine did not increase urinary creatinine, and that the feeding of creatin caused no increase in creatin excretion but was followed by a slight increase in eliminated creatinine.

The diet given our dogs consisted of unsweetened condensed milk, cracker meal, lard and water. Dog I took food with no apparent hunger and would at no time eat the prescribed amount, some days taking scarcely any. To this, in part is to be assigned the rapid loss in weight. Dog II took his food well and maintained constant weight.[2]

A study of the tables shows that ingestion of creatinine increased the creatinine output in the urine. The excretion of this substance is not quantitative in every instance, though at times this is approximated. There was no clear cut effect upon creatin excretion after giving creatinine. Following the ingestion of creatin there is no corresponding rise in creatin excretion; a slight rise in the creatinine output is suggestive but not convincing. Both creatinine and creatin appear to act as diuretics and the increase in nitrogen excretion on those days is possibly due to a washing out of excretory substances rather than to a conversion of creatin or creatinine into some other substance previous to excretion. The disproportion between the urinary nitrogen and the amount of substance administered suggests this explanation:

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[1] *Zeit. f. Physiol. Chem.*, 1909, lxii, 465.

[2] Both dogs were examined carefully at autopsy to ascertain if a collateral circulation had rendered the Eck fistula useless for our purpose. In Dog I no such condition could be detected, but in Dog II a minute aberrant branch of the splenic vein was found which in some slight degree might have diminished the effects of the operative anastomosis.

Dog I. Eck Fistula.

Date, 1910.	Body Weight, Kilos.	Amount of Urine, c.c.	Total N, gram.	Creatinine, gram.	Creatin, gram.	Amount Fed.
April 11	11.85	625	3.79	0.209	None	
12	11.81	410	2.41	.167	.040	
13	11.45	690	4.50	.364	.084	2.33 gm. creatin.
14	11.60	330	3.77	.251	.046	
15	11.50	340	2.81	.219	.027	
16	11.50	300	2.47	.135	.015	
17	11.30	420	5.46	.399	.032	
18	11.20	490	2.79	.172	.014	
19	11.35	840	6.29	.756	.043	0.60 gm. creatinine.
20	11.05	240	2.89	.166	.017	
21	10.80	420	4.99	.318	.067	1.2 gm. creatin.
22	10.68	550	3.57	.234	.063	
23	10.12	780	7.64	.694	.208	0.35 gm. creatinine.
24	10.07	425	3.62	.187	.161	

Dog II.

July 2	10.67	170	4.76	0.418	0.017	
3	10.35	130	3.06	.346	.078	
4	10.85	240	6.92	.381	.004	
5	10.65	160	5.87	.350	.041	1.11 gm. creatin.
6	10.70	115	2.58	.253	.002	
7	10.60	100	2.94	.288	.080	1.0 gm. creatin.
8	10.65	430	9.99	.507	.009	0.3 gm. creatinine.
9	10.60	180	4.69	.460	.000	
10	10.78	135	2.56	.360	.008	
11	10.72	160	4.96	.373	.076	
12	10.68	320	3.14	.326	.014	
13	10.71	175	3.14	.378	.004	
14	10.70	190	3.04	.363	.017	
15	10.76	420	3.31	.340	.008	
16	10.72	355	4.12	.387	.161	1.07 gm. creatin.
17	10.65	230	2.77	.324	.000	
18	10.68	160	3.89	.301	.010	
19	10.67	300	3.03	.486	.011	0.5 gm. creatinine.

[Compare the data for the 13th with the 21st day and those for the 19th with 23d of Dog. I.[1]] The creatin used in these experiments gave no reactions for creatinine. We are indebted to Dr. S. R. Benedict for creatinine made from this creatin. The amounts were computed from weights of substance on the basis of quantitative estimation by the Folin method.

[1]We are indebted to Drs. E. H. Poole and H. H. Janeway for operative procedures.

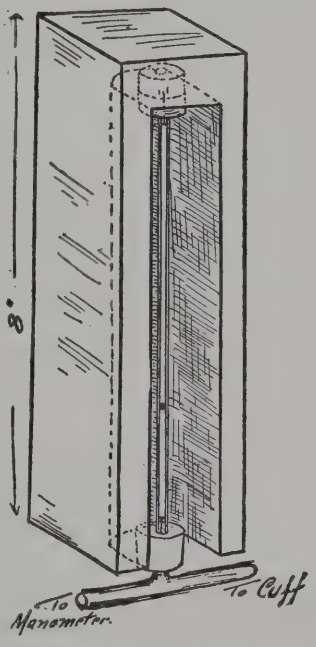
19 (544)

A blood pressure apparatus with pith-ball attachment indicating diastolic pressure.

By **NATHANAEL FEDDE.**

[*From the Laboratory of Physiology of the Cornell University Medical College.*]

Of the many factors governing blood pressure, the most important, namely the force of the heart beat, receives least attention. A knowledge of the systolic pressure is of value in warning of certain dangers, but gives no clue as to the amount of work the heart does. In order to estimate this we must know the difference between the pressure during the heart beats and that in the intervals. The maximum pressure is easily ascertained by any one of the numerous sphygmomanometers. Only a few have means, in any way satisfactory, for determining diastolic pressure, and these are prohibitive in price and of tremendous bulk. To overcome these obstacles we have devised a small instrument that can be attached to any sphygmomanometer. This consists in an air chamber communicating by means of a glass tube containing a pith-ball with the tube leading from cuff to manometer. Any increase of pressure in the cuff is equalized in the chamber by a rush of air passing the pith-ball and moving it. With a steady slow exhaust of air the ball moves exactly as the point of Erlanger's lever, but without the fling. An average pulse throws Erlanger's lever about 3 cm., while the pith-ball flies about three times that distance. The movements of the ball are interpreted just as the movements of the lever in Erlanger's instrument. That point of pressure is read at which the oscillations begin to fall off from the maximum.



20 (545)

Some desirable results following water drinking with meals.

By **P. B. HAWK.**

[*From the Laboratory of Physiological Chemistry of the University of Illinois.*]

In the continuation of our studies on the influence of water drinking at meal time, data have recently been collected as to the influence of this factor upon some of the activities of the gastro-intestinal tract. Particular attention has been given to the stimulation of gastric secretion, the activity of the pancreatic function, and to the course of intestinal putrefaction. As regards the stimulation of gastric secretion it has been found that the stimulation is directly proportional to the volume of water ingested. The activity of the pancreatic function measured by the fecal amylase (Wohlgemuth's method) was found to be increased during the water period. At the same time intestinal putrefaction, as measured by the indican content of the urine (Ellinger's method) was decreased. Absorption was also facilitated and the excretion of fecal bacteria lowered when large volumes of water were ingested at meal time.

21 (546)

Metabolism after hypophysectomy.

By **C. G. L. WOLF** and **E. SACHS.**

[*From the Department of Chemistry, Cornell University Medical College, New York City.*]

Three out of a series of respiration experiments on sixteen dogs, in which a part or the whole of the hypophysis was removed were reported.

They represented: (1) Extirpation of part of the anterior lobe and all of the posterior lobe. (2) Removal of the anterior lobe, leaving the pars intermedia and the posterior lobe. (3) Complete hypophysectomy.

The amount of gland destruction was determined by serial sections of the brain after the autopsy of the animals. Control experiments were made to determine the effect of the operative

procedures without removal of the gland. There was no effect on the carbon dioxide output.

In case 1 there was a distinct lowering of the carbon dioxide output, and a low level was established which persisted for sixteen days after the operation. At the end of this time the animal, which had previously been retaining nitrogen, was again in nitrogen equilibrium. On autopsy, an increase in fat and atrophy of the ovaries was observed. The weight of the animal had increased.

In case 2 there was a lowered carbon dioxide output, although marked emaciation followed the operation.

Case 3 lived but 48 hours after the operation. A respiration experiment was performed 24 hours after hypophysectomy. There was a markedly decreased carbon dioxide output, and an unusually low nitrogen output for the same period.

The apparatus used in these experiments was a modified Pettenkofer-Voit. The periods used for the determinations were six hours.

22 (547)

The influence of calcium and of sodium in M/10 solution upon the conductivity in nerve trunks.

By **DON R. JOSEPH** and **S. J. MELTZER.**

[*From the Department of Physiology and Pharmacology of the Rockefeller Institute for Medical Research.*]

In a previous communication before this Society[1] we reported that calcium chloride in an *M*/10 solution is capable of reducing or completely abolishing the direct and indirect irritability of frog muscles. The reduction or abolition is reversible; sodium chloride restores rapidly the lost irritability. It was further found that the primary action of calcium does not affect both forms of irritability in an equal manner; in a number of cases, especially under certain conditions of temperature and season, a comparatively small amount of calcium solution abolished completely the indirect irritability (from the nerve) while the direct muscle irritability still persisted in nearly its original intensity. From these experiments we concluded, among other things, that calcium af-

[1] Joseph and Meltzer, PROCEED. OF THE SOC. FOR EXPER. BIOL. AND MEDICINE, vol. vi, p. 104, 1909.

fects the motor nerve endings more readily than the muscle tissue; in other words, *calcium, like sodium, potassium, and magnesium exerts a curare-like action upon the motor nerve endings.* However, there was one link missing in the evidence in favor of the conclusion mentioned. The abolition of the indirect irritability might be due to the action of calcium upon the nerve trunk, and not upon the nerve ending. We have therefore studied the action of calcium chloride upon the nerve trunk in a short series of experiments. The results of this study form the subject of our present communication.

In our former studies the calcium solution was administered to the muscle by intravascular perfusion. For our present study bathing of the nerve in the solution was the method which had to be employed. A very small cup made of a section of glass tubing was filled with this solution, into which a loop of the sciatic nerve was immersed and kept down by a small pledget of absorbent cotton saturated with the same solution. The brim of the cup was slightly covered with vaseline to prevent the overflow of the solution. The section of the sciatic nerve between the cup and the gastrocnemius muscle was covered with a pledget of cotton saturated with Ringer. The same was the case with the lumbar plexus which was used for stimulation and kept on an appropriate electrode. The graphic registration of the contractions of the gastrocnemius muscle were obtained in the usual manner. The drum was turned by hand at arbitrary intervals. The stimulations were accomplished by single induction shocks (break) which at the beginning of the test gave a maximal contraction. Every few minutes the effect of a stimulation of the lumbar plexus was tested, comparing it sometimes with the effect of a similar stimulation of the part of the sciatic nerve peripheral to the cup.

In every experiment both legs were used at the same time: one for testing the effect of an $M/10$ calcium chloride solution and the other to study the action of an $M/10$ sodium chloride solution. Only the effect upon the conductivity was studied; the loop was never taken out of the cup to test also the effect of the solutions upon the irritability.

Ten experiments were made, nine of which gave the following uniform results which we shall state very briefly.

Primary action of calcium chloride.—In every experiment a time came when the conductivity of the nerve trunk became finally abolished; stimulation of the lumbar plexus gave no reaction, while stimulation of the distal part of the sciatic nerve brought out a good response. This, however, occurred only after prolonged bathing. Ninety minutes was the shortest period; in some cases it took 150 minutes and longer before all response from the lumbar plexus disappeared.

This result bears out our original conclusion. In our former experiments the indirect irritability disappeared after a few minutes exposure to the action of the calcium chloride. This could not have been due to the action of the calcium solution upon the nerve trunk since calcium is able to produce abolition of conductivity only after hours of bathing.

Reversible; restored by sodium chloride.—In every experiment the vanished conductivity came back after replacing the calcium solution by an $M/10$ solution of sodium chloride. The conductivity returned in a comparatively short time, probably in less than 15 minutes. After recovery, the lumbar plexus responded in a manner similar to that of the distal part of the sciatic nerve, which after several hours of exposure to the abnormal surroundings usually lost somewhat of its original irritability.

Primary action of sodium chloride.—It has been established already by Locke, and by Overton, that physiological salt solution does not affect the conductivity of the nerve trunk. Our experiments simply confirm these statements. After many hours of bathing of the nerve in an $M/10$ sodium chloride solution the lumbar plexus lost indeed some of its original irritability; but the loss was not greater than that of the distal section of the sciatic nerve which was kept covered throughout the experiment with cotton saturated in Ringer. We ought to add that the temperature of the laboratory during the period in which the experiments were carried out (November) was by no means low.

23 (548)

Observations on the nitrogen content of the succus entericus.

By **HERMAN O. MOSENTHAL, M.D.**

[*From the Laboratory of Biological Chemistry and from the Department of Medicine of Columbia University, at the College of Physicians and Surgeons, New York.*]

The succus entericus in these experiments was obtained by means of a "Thiry" fistula in dogs. The nitrogen content and the quantity of the succus entericus secreted by the entire small intestine of each dog in twenty-four hours was estimated by multiplying the amounts collected from the fistula in a given period by two factors: first, by the figure necessary to bring the number of hours up to twenty-four, and second, by the figure required to compensate for the length of the dog's small intestine, as determined at autopsy.

Summarizing the protocols, the following average figures are obtained:

	Number of Observations.	Weight of Dog, kilos.	N Intake, gms.	N of Succus Entericus, Calculated for 24 Hours, gms.	N of Feces, gms.	N of Urine, gms.	N of Succus Entericus as Per Cent. of:		
							N Intake.	N Feces.	N Urine.
Dog 1	3	13.9	8.8	3.1	0.8[1]	8.0[1]	35	383	39
2	5	11.7	6.7	2.4	0.6[1]	5.0	34	393	47
3	15	7.0	7.4	1.5	0.7	5.6	21	225	27
4	14	7.1	4.3	1.9	0.3	3.2	43	607	59

Conclusions.—Nitrogen to the amount of about 35 per cent. of the food nitrogen of a mixed diet, is daily secreted in the succus entericus in dogs. Of this quantity, an amount equal to about 10 per cent. of the food nitrogen is excreted in the feces and an amount equal to about 25 per cent. of the food nitrogen is reabsorbed. The amount of reabsorbed nitrogen-containing material is considerably larger if the bile and pancreatic secretion are included.

The metabolic significance of this reabsorption can only be surmised; that it is probably of great importance is indicated by the fact that in the experiments presented, so large an amount of nitrogen, *i. e.*, equal to approximately 25 per cent. of the nitrogenous part of the food, is concerned.

[1] The value is estimated; was not determined.

24 (549)

The relation of the adrenals to tuberculin poisoning.

By **JAMES P. ATKINSON** and **CHAS. B. FITZPATRICK.**

[*From the Chemical and Research Laboratories. Department of Health, City of New York.*]

A paper which we read before this society in the spring of 1910 showed that "old" tuberculin or the filtered fluid from a culture of *B. tuberculosis* caused a marked arterial depression in the dog when injected into the femoral vein. This same tuberculin if heated above 105° lost this power of depression.

We have recently found that commercial adrenalin preparations and saline extracts of the experimental dog's freshly removed adrenal gland prevented this depression when mixed with tuberculin and the mixture injected intravenously.

Samples of tuberculin, which caused marked drops in the blood pressure, when mixed with a sufficient amount of an emulsion of the dog's own adrenals and injected intravenously, caused no fall in the blood pressure, thus showing an antagonistic action between tuberculin and the adrenals. The intravenous injection of samples of tuberculin containing the active depressor substance, after the adrenals had been removed, caused a drop with a more gradual recovery to the previous pressure level, than when the adrenals had been left intact. The intravenous injection into the dog with both adrenals removed of an emulsion of its own adrenals caused a return of the blood pressure to and above the normal level.

Further light is apparently thrown on the nature of the poisons of the tubercle bacillus as found in tuberculin by the following observations.

A. Four tuberculous guinea pigs were injected with a mixture of .5 c.c. of "P. D. & Co. Adrenaline Chloride 1–1000." Three of the pigs died within 18 hours and one within 36 hours. One control, a tuberculous pig, receiving .5 c.c. tuberculin without adrenalin died within 18 hours. The other control, a normal guinea pig, receiving .5 c.c. of the adrenalin chloride "P. D. & Co. 1–1000" did not apparently suffer any harmful results or show any noticeable toxic symptoms.

Four tuberculous guinea pigs injected with a mixture of .5 c.c.

tuberculin and 2 c.c. of a 1/100 dilution of "P. D. & Co. Adrenalin Chloride 1–1000" died within 18 hours. The control, a guinea pig, infected with *B. tuberculosis*, with enlarged glands, was injected with 2 c.c. of the same diluted adrenalin solution and did not show any toxic symptoms and lived.

B. .5 c.c. of the same tuberculin used in the previous experiments heated from 106° C. to 110° C. for 1 hour killed a tuberculous guinea pig within 18 hours.

We have studied the nature of tuberculin poisoning further by injecting tuberculin into dogs after one and both adrenals have been removed. The characteristic kymograph reaction of a tuberculin injection into normal dogs is a quick fall followed by an almost equally quick rise in pressure to the previous level.

The injection of a depressing dose of tuberculin into the dogs with the adrenals removed caused a marked drop with a much delayed response in the return to the previous pressure level.

Repeated successive depressing doses, varying from .5 c.c. to 5 c.c. for each dose, of samples of tuberculin injected intravenously into several large dogs which had had both adrenals removed did not cause a drop to the base line or immediate death, but the dogs lived from 2½ to 3 hours after the removal of the adrenals.

The average life of a dog after both adrenals have been removed is about 40 hours. Little or no ether was required after both adrenals had been removed to keep the dogs anesthetized. Clotting, which after the intravenous injections of samples of tuberculin in several doses, varying from .5 c.c. to 5 c.c. for each dose, had always seriously interfered with our work with the kymograph, was entirely absent after the adrenals had been removed even when tuberculin had been repeatedly injected.

We have repeatedly injected normal dogs in order to sensitize them with 5 c.c. of crude tuberculin without apparent injury or noticeable toxic effects.

These results indicate:

A. That tuberculin is a complex substance consisting of at least two poisons, one a blood pressure depressor destroyed by heat and antagonized by adrenalin; the other a substance characterized by its fatal effects on tuberculous guinea pigs when injected sub-

cutaneously and by its stability, which resists heating to 110° C, and which is not neutralized by adrenalin.

B. It is possible that we have evidence here that the absence or the abnormal diminution of the adrenal secretion permits some of the pathogenetic action of the products and extracts of the tubercle bacillus and that their administration mixed with the whole adrenal or some part of it, in a measure overcomes their deleterious action.

C. It is possible that the neutralizing value of blood mixed with tuberculin or some other appropriate adrenal antagonist might be a valuable index of the functional condition of the adrenal glands.

25 (550)

Comparison of the blood-flow in the hands in a case with lesion of upper motor neurones (birth palsy) and in a case with lesion of lower motor neurones (infantile paralysis).

By **G. N. STEWART.**

[*From the Department of Experimental Medicine, Western Reserve University.*]

The blood flow was calculated from the formula

$$\varphi = \frac{H}{T - T'} \times \frac{1}{S}$$

where φ is the quantity of blood flowing through the hand in the period of observation, H the heat given off to a calorimeter containing the hand, T the temperature of the arterial blood coming to the hand (taken as rectal temperature),[1] T' the temperature of the venous blood leaving the hand (taken as the average temperature of the water in the calorimeter[2]) and S the specific heat of blood. Before being put into the calorimeter the hand was immersed for a sufficient time (usually ten minutes) in a large

[1]Observations since made on the actual temperature of the arterial blood show that in a healthy man the rectal temperature is about half a degree above that of the blood coming to the hand under the conditions of the experiments. The temperature of the arterial blood is arrived at by determining that temperature of the calorimeter at which the hand neither loses nor gains heat.

[2]That this assumption is approximately correct for a certain range of bath temperature has been shown by actual measurement of the temperature of blood issuing from one of the veins of the hand, with suitable precautions to render the loss of heat as small as possible.

bath containing water at the same temperature as that in the calorimeter in order that T' might approximate to the temperature of the calorimeter. All thermometers used were, of course, compared with a standard thermometer. In the cases observed, the two hands were simultaneously immersed in two precisely similar calorimeters. The volume of the hand enclosed in the calorimeter was determined by afterwards immersing the hand up to the proper level in a lipped beaker previously filled with water and measuring the overflow, or by immersing it in a vessel provided with a gauge.

Case 1.—Man æt. 46, weight 160 lbs., height 5 ft. 10 in. Marked contracture of the flexors of left wrist, with spastic paralysis of hand and fingers; also some deformity of left foot. A history was obtained indicating gradual onset of the contractures. At the age of two deformity was noted in the left hand and slight deformity in the left foot. The condition gradually got worse. The general health is good and the man is able to work as a telegraphist. There is some control of the movements of the left hand. He can extend the fingers and flex them but not normally. He uses the hand to a certain extent in his occupation. It is markedly smaller than the right. The pulse at the wrist is quite distinct. The hand feels as warm as the right one. The case was diagnosed as birth palsy by Dr. Henry O. Feiss, to whom I am indebted for the opportunity of studying it and the other case.

PROTOCOL OF EXPERIMENT.

Three liters of water in each calorimeter.

3.39 P.M. Both hands immersed in bath at 27.8°.

3.45 Temperature of bath 27.4°.

3.49 Left hand put into calorimeter A, right into B. Patient standing and hands hanging down.

	A.	B.
3.51	27.63	27.60
3.52	27.68	27.60
3.53	27.72	27.66
3.54	27.81	27.73
3.55	27.89	27.81
3.56	27.95	27.95
3.57	28.00	28.01
3.58	28.09	28.13
3.59	28.20	28.29
4.00	28.22	28.33
4.01	28.29	28.40

At 4.01 the hands were rapidly withdrawn and the final reading taken after vigorous stirring. Variations in the previous readings due to inadequate stirring or accidental contact of the fingers with the thermometer are thus controlled.

RATE OF COOLING OF CALORIMETERS AFTER WITHDRAWAL OF HANDS.

	A.	B.	Room.
4.18	27.94	28.10	20.1
4.27	27.85	28.00	
4.36	27.77	27.91	19.5

Volume of right hand in calorimeter 370 c.c.

Volume of left hand in calorimeter 330 c.c.

Pulse rate in standing position 84. Rectal temperature 37.4.

Taking the water equivalent of hand and calorimeter together as 300 grm. and the loss of heat by the calorimeter as 0.01° per minute, we get for the left hand H = 2,508 small calories; T = 37.4; T' (average temperature of the calorimeter) = 27.96. Taking the specific heat of blood as 0.9, we get

$$\varphi = \frac{2508}{9.4} \times \frac{10}{9} = 296 \text{ grm. blood}$$

in ten minutes, *i. e.*, 9.0 grm. blood per minute per 100 c.c. of hand.

For right hand φ = 354 grm. blood in ten minutes, *i. e.*, 9.6 grm. blood per minute per 100 c.c. of hand.

Case 2.—A boy 9 years and 4 months old. Normal delivery, no instruments used. At the age of one month the child had convulsions. At 6 months the mother noticed disability in the left arm and left leg. The arm has since become flaccid, and the whole arm is atrophied. The upper arm can only be raised level with the shoulder. Some power of flexion of the forearm at the elbow but hardly any power of rotation. The hand is held pronated and everted toward the ulnar side and the fingers are extended. They are small and cold. There is some power of flexion of the middle, ring and little fingers but very little in the case of the thumb and index finger. Movements at wrist very slight.

Diagnosis: infantile paralysis.

PROTOCOL OF EXPERIMENT.

At 2—11—30″ P.M. Both hands put in bath at 26.6°.

2.19 Temperature of bath is 26.5°.

At 2.19 Put hands into calorimeters, each containing 2,800 c.c. water. (These were somewhat smaller calorimeters than those employed for case 1.)

Patient standing.

	Calorimeter containing left hand.	Calorimeter containing right hand.
2.20	26.32	26.40
2.21	26.36	26.45
2.22	26.40	26.55
2.23	26.53	26.70
2.24	26.54	26.80
2.25	26.54	26.85
2.26	26.55	26.95
2.27	26.60	27.05
2.28	26.61	27.10
2.29	26.65	27.20
2.30	26.65	27.30

The last readings were taken after vigorous stirring immediately after removal of the hands from the calorimeters.

COOLING OF CALORIMETERS AFTER WITHDRAWAL OF HANDS.

	Left.	Right.	Room.
2.43	26.60	27.20	23.4

Pulse 95 in standing position. Rectal temperature 37.6°.

Volume of left hand in calorimeter 170 c.c.

Volume of right hand in calorimeter 210 c.c.

The hands being small, portions of the arms were included.

φ = 111 grm. blood in ten minutes for left hand, *i. e.*, 6.5 grm. per minute per 100 c.c. of hand.

φ = 306 grm. blood in ten minutes for right hand, *i. e.*, 14.5 grm. per minute per 100 c.c. of hand.

Conclusion.—In the hand whose lower motor neurones were not involved in the lesion producing the paralytic condition the blood-flow per unit volume of hand substance is scarcely inferior to that in the normal hand.

In the hand a lesion in whose lower motor neurones is responsible for the paralysis, the blood flow per unit volume of hand substance is 2½ times less than in the normal hand. The difference in the amount and condition of the muscular tissue is one important factor in causing the difference in blood flow in the two conditions.

26 (551)

Edema formation in guinea pigs in chronic experimental uranium nephritis.

By **ERNEST C. DICKSON.**

[*From the Pathological Laboratory of Cooper Medical College.*]

In a series of experiments performed on guinea pigs during the past two years, with the purpose of confirming the findings in experimental chronic nephritis which I have previously reported,[1] some interesting observations in edema formation have been made. Twenty-one animals received subcutaneous injections of an aqueous solution of uranium nitrate, as follows: six received numerous injections of 0.5 m.grms. at frequent intervals; eight received several injections of 5 m.grms. at longer and irregular intervals; and seven received one or more injections of 10 to 15 m.grms. Four animals died within two weeks after the first injection, and can be excluded from the chronic nephritis series. The remaining seventeen survived for from three to twenty-three months after the first injection, and all showed kidney lesions of a chronic nature, similar to those which I have previously described.

In Group I, which received the numerous small doses, two

[1] *The Archives of Internal Medicine*, June, 1909, vol. iii, No. 5.

animals which had received 26 and 80 injections, respectively, survived for seven and twenty-three months, and at autopsy showed no edema. Two which had received 47 and 70 injections, respectively, survived for twelve and nineteen months, and at autopsy showed marked ascites, over 30 c.c. of fluid being collected in the latter case. The remaining animal which received 86 injections survived for twenty-three months, and at autopsy showed marked ascites, and double hydrothorax. In all but one case in this series, death occurred in from two to five days after the last injection.

In Group II, four animals which died within four months after the first injection, showed no edema. One which received 10 injections of 5 m.grms. each, during fifteen months, died two days after the last injection, and showed marked subcutaneous edema, but no accumulation of fluid into the serous cavities. This animal increased 50 grms. in weight during the last two days of life. One animal which received eight injections during thirteen months, died five days after the last injection, and showed moderate subcutaneous edema, marked ascites, double hydrothorax, and hydropericardium. Still another which received four injections during eight months, died eleven days after the last injection, having increased 125 grms. in weight during the last five days of life, and at autopsy showed marked subcutaneous edema, and marked ascites, over 120 c.c. of ascitic fluid being collected. The remaining animal of this series received seven injections during fourteen months, died eight days after the last injection, and showed moderate ascites.

In Group III, one animal which received two injections of 10 m.grms. and one which received a single injection of 15 m.grms. died within four months, and showed no edema. One which received 30 m.grms. in three doses, died in sixteen months, eleven days after the last injection, and showed ascites, double hydrothorax, and hydropericardium. The remaining animal received 20 m.grms. in three injections, died in sixteen months, five days after the last injection and showed moderate subcutaneous edema, and moderate ascites.

In the complete series of seventeen animals which survived for longer than three months, seven which died before the eighth

month showed no edema, and of the ten which lived for longer than eight months, nine showed definite, and in some cases, marked edema. In four of the nine cases there was marked subcutaneous edema, which in three cases was associated with effusion into the serous cavities. All of the cases of subcutaneous edema occurred in Groups II and III, where relatively large doses of the drug were administered.

In none of the cases was any attempt made to induce edema formation by forcing water. The animals were fed upon a mixed diet in which was a plenty of greens, and they obtained all their water from the green food. Since death occurred in every case but one within eleven days after the last injection, and during the resulting acute intoxication, it would seem that the edema was a true renal edema, and not due to stasis resulting from a broken down heart. There can be little doubt that the prolonged action of the uranium nitrate upon the blood vascular system had so damaged it, that it was unable to withstand the strain of the plethora which was produced by the inability of the kidney to excrete water during the terminal attack of acute nephritis.

27 (552)

The action of infundibulin upon the mammary secretion.

By **ISAAC OTT** and **JOHN C. SCOTT.**

[*From the Physiological Laboratory, Medico-Chirurgical College of Philadelphia.*]

In the goat we have found in the early nursing period that infundibulin (the active principle of the posterior part of the hypophysis), when injected into the vein of the ear, rapidly and greatly increased the secretion of milk. The nipple had a cannula inserted into it, and a water aspirator produced the suction necessary to empty the udder. The milk before and after the injection was caught in a graduated flask and measured every five minutes. The following experiment will given an idea of the activity of the infundibulin:

GOAT—RIGHT NIPPLE.

2.25 P.M.			
2.30 "	.....	4 drops milk.	
2.35 "	.....	5 "	"
2.40 "	.....	5 "	"

2.41 P.M. 5 drops of infundibulin by the vein.
2.45 " .....405 drops milk.
2.50 " ..... 15 " "
2.55 " ..... 22 " "
3.00 " ..... 12 " "
3.05 " ..... 8 " "
3.10 " ..... 4 " "
3.11 " 5 drops of infundibulin by the vein.
3.15 " ..... 75 drops milk.
3.20 " ..... 15 " "
3.25 " ..... 15 " "
3.30 " ..... 7 " "
3.35 " ..... 6 " "
3.40 " ..... 5 " "
3.45 " ..... 4 " "

Care was taken to thoroughly empty the udder both by aspiration and by rhythmic external compression of the gland. The intra-venous injection of infundibulin starts the flow in about one minute from the beginning of the injection, and it reaches its height in four minutes, after which it rapidly falls to normal.

28 (553)

The galactagogue action of the thymus and corpus luteum.

By **ISAAC OTT** and **JOHN C. SCOTT.**

[*From the Physiological Laboratory, Medico-Chirurgical College of Philadelphia.*]

In experiments upon the goat with the glands containing internal secretions, we have found the corpus luteum, pineal body and thymus increased the quantity of milk fourfold in five minutes. The ovary minus corpus luteum had no effect. Infundibulin is still the most powerful galactagogue, increasing the secretion of milk one-hundredfold. The amount of butter fat was about the same in the augmented secretion by thymus, corpus luteum, and infundibulin, but occasionally it was increased.

SCIENTIFIC PROCEEDINGS.

ABSTRACTS OF THE COMMUNICATIONS.

Forty-second meeting.

Cornell University Medical College. February 15, 1911.

29 (554)

Cell Division and Cell Regeneration. I. Uronychia transfuga.

By **GARY N. CALKINS.**

[*From the department of Zoölogy, Columbia University.*]

1. Although many experimenters have studied the regenerative power of different kinds of protozoa no one has observed the variations in this power at different age periods after division.

2. *Uronychia transfuga* is a marine hypotrichous ciliated protozoon about 100μ in length. It may be cut in any plane with a fine pointed scalpel and the pieces will live for several days, thus showing a hardiness which makes it a good subject for experimentation.

Under conditions of culture in the laboratory (at Roscoff, France) the cell divides about once in 36 hours, the period between divisions being marked by definite changes in size of the cell and in arrangement of the nuclear elements (macronucleus and micronucleus).

3. Experiments to test the regenerative power were made upon cells immediately after division; on cells from 12 to 18 hours after division; on cells immediately before division; and on cells during division. The cells, in all cases, were cut with a scalpel under a microscope, full records of 75 experiments being kept.

4. If cut immediately after division the fragments continue to live for at least three days, but that fragment alone regenerates which contains macronuclear material and the micronucleus. The other fragment contains a variable amount of macronuclear material but no micronucleus. In two of the thirteen successful experiments on cells in this stage, neither fragment regenerated.

In other cases regeneration of the micronucleus-holding fragment was completed in from twelve to eighteen hours.

5. In cells cut from twelve to eighteen hours after division and after full size is attained, the results are similar to those on cells immediately after division. The micronucleus-holding fragment alone regenerates, the other fragment, containing only macronuclear material lives for several days but fails completely to regenerate. This result was obtained in twelve different cases.

6. If cut just prior to division both fragments regenerate within 24 hours, the one fragment containing only macronuclear material, the other, containing both macronucleus and micronucleus. The latter regenerates more rapidly than the former. One cell regenerates without a micronucleus.

7. If cut during the early stages of division three completely regenerated cells result, one of which (the fragment cut off) contains no micronucleus. Division continues in the original plane, the result being one normal cell and one minute cell perfect in all respects save size. Regeneration of the micronucleus-holding cells is more rapid than that of the third fragment. This result was obtained in fourteen cases.

If the cells are cut during the later phases of division the results are similar to those obtained by cutting immediately after division. One fragment regenerates, the other fails. This result was obtained in seven out of eight experiments on cells in this stage, the other one gave regeneration of both fragments.

8. The experiment shows a considerable variation in the power to regenerate in this form; this power is lowest immediately after division, but it gradually increases with age after division until it reaches a maximum immediately before the next division and during the early phases of division.

During division it decreases to a minimum in the later stages and immediately after division.

9. The results might be interpreted by the assumption of a specific substance, possibly enzymatic in nature, which accumulates with age of the cell until a condition analogous to saturation is reached. With the formation of the new cell-organs this substance, it may further be assumed, is exhausted and regeneration

is impossible save with the full complement of cell organs (macro- and micronucleus and cell protoplasm).

10. Experiments in cutting *Paramecium caudatum* were also made. Here regeneration of the cell does not occur save under exceptional conditions which I shall report at length upon later. A frequent result is the formation of monsters with from two to fourteen mouths; an abnormality due to some derangement of the cellular mechanism through the removal of a small portion of the cytoplasm.

30 (555)

Iodine as a skin disinfectant in animal surgery.

By **H. W. MAYES.**

[*From the Department of Physiology and Biochemistry, Cornell University Medical College, Ithaca, N. Y.*]

In experimental physiology where it is necessary that the animal be allowed to recover after the removal of an organ or the establishment of a lesion, the operative procedure is carried out under full anæsthesia and with aseptic or antispetic precautions as in human surgery. The preliminary disinfection of the skin by scrubbing with soap and water and the subsequent washing with bichloride, carbolic or alcohol, takes considerable time and in most cases must be done after the animal is anæsthetized; besides where the operative field includes the head or face there is always danger of the eyes being accidentally injured by the irritative fluids. Then again, after operation everyone has experienced the difficulty of keeping the dressing properly applied to the wound which must be protected from outside contamination unless the animal be kept in aseptic surroundings—a condition practically impossible in most laboratories. Any method, therefore, which will materially save time and trouble and at the same time not increase the risk is particularly desirable in animal surgery and such a method, I believe, is to be found in the use of iodine as a skin disinfectant.

Iodine was first applied in human surgery about fifty years ago by Bryant[1] and Boinet[2] and recently it has come into vogue

[1] *Brit. Med. Journ.*, 1910, I., p. 1003.

[2] *The Lancet*, 1910, CLXXIX., p. 1888.

again. The results are claimed to be eminently satisfactory but there may be objections to its application to the comparatively sensitive skin of the human subject on account of its irritating qualities which do not obtain in the case of animals.

The method adopted by me, which is a modification of that employed by Grossich, is as follows: Immediately before the animal—*e. g.*, cat or dog—is put into the ether chamber the hair is cut short and the skin shaved dry along the line of the intended incision. Tincture of iodine (U.S.P. 7.5 per cent. in 95 alcohol) is painted on and a little way around the shaved area with a camel hair brush and the anæsthetic is administered. When the animal is fully under (with ether this usually takes ten to fifteen minutes) and placed on the table a second application of the iodine is made a minute or two before the skin is incised, and a third, after the stitches are in, when the operation is finished. No dressing of any kind is applied to the wound but the day following the operation a fourth application of the tincture is made. No further treatment is necessary,

During the last four months many operations have been performed in this laboratory on a variety of animals—rabbits, cats, dogs, sheep, raccoons, opossums, etc., and in every case where the above procedure has been adopted healing has been by first intention and rapid.

31 (556)

The Diagnosis of Abortive Cases of Poliomyelitis by the Demonstration of Specific Antibodies.

By **JOHN F. ANDERSON** and **WADE H. FROST.**

[*U. S. Public Health and Marine Hospital Service, Washington, D. C.*]

The occurrence of abortive cases of poliomyelitis has already been established upon clinical and epidemiological ground. Netter and Levaditi[1] have given the only specific proof of an abortive case of poliomyelitis in a human being by demonstrating, in the serum of such a case, immune bodies capable of neutralizing the

[1] Netter, A., & Levaditi, C., *Compt. Rend. de la Soc. de Biol.*, vol. 68, 1910, pp. 855–857.

virus of poliomyelitis *in vitro*. Their case was a child which suffered a clinically obscure, mild illness about the same time that another child of the same family suffered a frank attack of poliomyelitis.

We have undertaken a similar demonstration in nine suspected abortive cases of poliomyelitis selected from a much larger number observed during an epidemic of acute anterior poliomyelitis in Iowa in the summer of 1910. Special interest is attached to these cases because of the mildness of their symptoms, the frequency of similar cases and their epidemiological relation to cases of frank poliomyelitis.

The sera of these nine cases were tested for immune bodies as follows: One half cubic centimeter of each serum was mixed with an equal volume of a 5 per cent. emulsion (filtered through paper) of fresh spinal cord from a monkey in the acute paralytic stage of poliomyelitis following inoculation with virus (M.G.) kindly furnished by Dr. Simon Flexner, of the Rockefeller Institute. To each mixture was added 0.1 c.c of fresh serum from a normal adult. As controls, we used the serum of a frank case of poliomyelitis and of a normal adult. The mixtures were allowed to stand 1 hour at 27° C. and 20 hours at 15° C. One half cubic centimeter of each mixture was then injected intracerebrally into a monkey (*Macacus rhesus*). The two control monkeys receiving the mixture of normal serum and virus developed typical poliomyelitis on the tenth and twelfth day respectively. Three of the monkeys receiving the serum from suspected abortive cases also developed poliomyelitis.

The monkeys receiving the serum from the other six suspected abortive cases, and from the frank case of poliomyelitis have all remained well (78 days).

We then repeated the test, using the serum of the three suspected abortive cases which had failed to show immune properties in the first experiment; and for controls, using five specimens of normal human serum (three adults and two children). In this series we altered the proportion of serum and virus to the extent that we used a *one per cent.* emulsion of fresh spinal cord, all other conditions remaining the same. In this series poliomyelitis developed in only two of the controls, and in one of the monkeys

receiving the serum of a suspected abortive case of poliomyelitis, indicating presumably a certain degree of germicidal power even in *normal* human serum.

To compare this germicidal property of normal serum with that shown by the sera from the six suspected abortive and two paralytic cases in series 1, we inoculated a third series of monkeys. In this series we tested the three normal sera which had shown neutralizing properties in series 2 using a *5 per cent.* emulsion of virus, thus repeating exactly the conditions of series 1. All three monkeys developed typical poliomyelitis in 10 to 14 days.

The sera of six out of nine (66.7 per cent.) suspected abortive cases of poliomyelitis have shown, when tested against the virus of poliomyelitis, a germicidal property greater than that shown by any one of six normal sera similarly tested. We interpret this as establishing, to a reasonable certainty, the diagnosis of poliomyelitis in these six cases, and strongly confirming the same diagnosis in a larger group of clinically similar cases observed in connection with the above.

Attention is called to the epidemiologic and prophylactic importance of establishing, by close study of such cases, some clinical criteria for their better recognition.

32 (557)

Pure cultures of parasitic amebas on brain-streaked agar.

By ANNA W. WILLIAMS.

[*From the Research Laboratory, Department of Health, City of New York.*]

This work was begun with the attempt to obtain cultures of bacteria-free amebas in crushed rabies brains, the idea being either that the amebas might take up the rabies organisms as food or that they might free the rabies organisms from their host cells and thus bring about, in some way, a culture of the latter, as Clegg reports having done with the *B. leprœ.*

Four cultures of amebas were selected for a first trial, 3, presumably parasitic (type, *Entameba coli*), the fourth, a saprophyte (*Ameba lirnax*). Of these four, only one, a culture from a case of

human amebic dysentery, grew with comparative ease and some abundance on agar plates streaked with rabies brains.

I then tried normal brains as controls and found that this strain of ameba grew with almost equal ease on them; so that the problem was changed for the time from that of a possible aid in determining the nature of the Negri bodies to that of the growth of pure cultures of amebas.

Only four investigators (Kartulis 1893, Casagrandi & Barbagallo 1897, Tsugitani 1898) have claimed to obtain amebas growing free from other living organisms and none of these have been quite clear in regard to their technic nor have they apparently grown their organisms for more than two or three culture generations, which, as controls show, does not rule out the factor of subsistence upon food stored up in the amebas themselves.

The majority of investigators who have tried to obtain pure cultures of amebas all make emphatic statements as to the impossibility of obtaining such cultures by any methods tried.

The present work, the details of which will be given in the full report, may be divided into 3 parts: (1) Obtaining living amebas free from other living organisms, (2) Obtaining sterile brain tissue, (3) Making successive transplants of amebas and brain tissue and proving that every transplant is free from other living organisms. Each step requires many controls.

In obtaining living amebas free from other organisms a method has been used which has been employed successfully by other investigators, differing only in detail. The stock culture of ameba and bacterium is planted in small amount in the center of a nutrient agar plate from which the water of condensation has been dried. On such a plate at 25° to 30° C., in 48 hours, the amebas usually spread beyond the bacteria. If they do not, a drop of normal salt solution will help them do it in another 24 hours, and there is then a wide-spreading zone of motile amebas minus bacteria. Platinum loopfuls of these edge amebas are transplanted to a series of agar plates, and thoroughly controlled for presence of bacteria.

The brain tissue is obtained by removing under strictly aseptic precaution the cerebrums of normal rabbits or guinea pigs, dropping them into 5 per cent. carbolic acid for one to two minutes, then placing them on agar plates and removing Ammon's horns

and corpus striatums which are spead over a series of fresh agar plates, and put in the thermostat for 24 hours. It is peculiarly important to have conditions such as to insure sterility of this tissue medium because of the possibility of the amebas using up any organism that might get into the plates before it could be detected.

This crushed sterile brain is now spread in small amounts over the plates containing the amebas, and placed, some in thermostat, and some at room temperature. If in 24 hours the amebas show slight or no growth as may be the case in the first culture generations, a loop or two of sterile normal salt solution may be added, then in 24 hours at 36° C. or 3–5 days at 20° C., there is generally an abundant growth.

To rule out the presence of extraneous organisms, at each transfer three or four plates are made. To one is added sterile normal salt solution, to another sterile broth, to another sterile blood, one is left simply with the transplanted material, and to the others are added the fresh sterile brain tissue for carrying on the culture. In the second or third culture generations, the cultures transferred on blood or broth or normal salt or plain agar encyst or die out. Spreads, making an added important control, have been made at practically every transfer and usually stained in three different ways. Two other strains of parasitic amebas are being tried and they seem to be growing, though it is too soon to make a positive statement. Liver tissue and spleen tissue are being tried, and the amebas seem to be growing on the liver tissue and not on the crushed spleen.

On the brain-streaked agar plates the one strain of ameba is now growing abundantly in the fourteenth culture generation, with normal brain and in the twentieth culture generation with rabies brain, grown at 36° C. and transplanted every 2–5 days.

33 (558)

A comparison of the growth of sarcoma and carcinoma cultivated in vitro.[1]

By **R. A. LAMBERT** and **FREDERIC M. HANES.**

[*From the Department of Pathology, College of Physicians and Surgeons.*]

We have used in this study a rat sarcoma, a mouse sarcoma and a mouse carcinoma, transplantable tumors of a high degree of virulence. The technique has been practically the same as that employed by Burrows in the cultivation of the tissues of chick embryos and subsequently by Carrel and Burrows in the growth of various mammalian tissues; that is, plasma was obtained by centrifugalizing fresh blood under conditions that prevent coagulation and allowing it to clot in hanging drops which contained small pieces of tumor tissue. These preparations were incubated at 37° C. We have found that rat sarcoma grows in both rat plasma and mouse plasma, and that this is also true of mouse sarcoma. The growth in both instances, however, seems to be more vigorous and of longer duration when homologous plasma is used.

For the character of the growth in vitro, a description of the growth of mouse sarcoma in mouse plasma will suffice. The edges of a piece of sarcoma embedded in plasma are at first fairly uniform in thickness and the piece of tissue is dense and opaque. After twelve hours of incubation at 37° numerous elongated cells project from all sides of the tissue and these wander out into the surrounding plasma by amœboid motion. The throwing out of pseudopods and the associated streaming of the protoplasm can be seen quite beautifully under the microscope. As the cells wander from the original piece of tissue, it becomes less dense and we have observed very frequently the reduction of the original piece of sarcoma tissue to only a fraction of its initial size. Indeed it may become after several days entirely resolved into its component cells, which wander further and further toward the periphery of the plasma. These migrating cells rapidly fill themselves

[1] This investigation has been conducted under the George Crocker Special Research Fund.

with small droplets of fat. This amoeboid wandering of cells is a striking phenomenon in the cultivation of sarcoma in vitro but mitotic division of these cells is frequently seen in stained preparations. "Ring" formation, as described by Harrison, is often observed especially when mouse sarcoma is cultivated in rat plasma. Within the ring where fibrin is absent the cells are seen growing along the cover glass.

The growth of carcinoma in vitro differs quite markedly from that of sarcoma. At the end of eighteen or twenty-four hours there is noted around the piece of incubated tissue a narrow fringe of polygonal cells with large, distinct nuclei. This change is associated with a general flattening out of the specimen. During the next few days this fringe becomes a wide sheet of cells, spread out in a single layer, surrounding the original piece. The edge of this sheet of cells presents an irregular protoplasmic border with moving processes,—a picture almost identical with that described by Harrison for the growth of the epithelium of frog embryos cultivated in frog's lymph. In some preparations groups of cells invade the plasma at certain points, forming what might be termed "alveoli," with the fibrin network as a stroma. In addition to the growth just described, there may be, particularly during the first twenty-four hours, a migration of irregularly shaped cells, similar in type to those seen in sarcoma. Further study will be necessary to determine whether these are carcinoma cells or stroma cells. In stained specimens of growing carcinoma, we have observed mitotic figures at various periods up to the fifth day of incubation. We have not studied specimens of longer duration.

This study seems to demonstrate how closely the character of the growth of tumor cells in the body may be simulated when these tissues are cultivated outside the body.

34 (559)

A respiration calorimeter of the Atwater-Rosa-Benedict type designed for use with dogs and children; with demonstration.

By **H. B. WILLIAMS.**

[*From the Physiological Laboratory, Cornell Medical College, New York City.*]

The apparatus demonstrated is in many respects a miniature of the calorimeters of this type which have been constructed at the Nutrition Laboratory in Boston. The writer wishes to acknowledge his great indebtedness to Dr. Francis G. Benedict, director of that laboratory, for his invaluable assistance in working out the problem of a small calorimeter.

In order to measure with a satisfactory degree of precision the gaseous and energy metabolism of infants and small animals, it has been necessary to introduce some special modifications. As a detailed description of these modifications with a report of the control tests of the apparatus will be communicated within a short time, particular mention need not be made at present.

35 (560)

The chemical and energy transformations in the dog after the ingestion of different quantities of meat.

By **H. B. WILLIAMS, J. A. RICHE** and **GRAHAM LUSK.**

[*From the Physiological Laboratory, Cornell Medical College, New York City.*]

A dog weighing about 13.8 kilograms was given on different days, 700 grams of meat and 1,200 grams of meat at the noon hour. In the morning the metabolism was determined for an hour, while the dog slept quietly in the respiration calorimeter. The minimum or basal metabolism thus determined was found to be about 25 calories per hour. After the ingestion of meat at noon, the animal was again placed in the respiration calorimeter, and the hourly metabolism determined. The results are given in the following table.

HEAT PRODUCTION AFTER MEAT INGESTION.

700 grams meat at noon.

	A. M. 9.45–10.45	P. M. 12.45–1.45	1.45–2.45	2.45–3.45	3.45–4.45	4.45–5.45	5.45–6.45	6.45–7.45
Calories found	25.06	30.78	35.07	33.98	34.09	33.87	33.51	33.49
Calories calculated	26.32	31.33	35.16	33.62	32.41	32.49	31.88	33.45
Grams urinary N.....	0.234	0.591	0.812	0.974	1.52	1.55	1.54	1.25
No. of experiments	(3)	(2)	(1)	(2)	(1)	(1)	(1)	(1)

1,200 grams meat at noon.

Calories found.....	25.06	33.28	33.96	41.52	39.59	38.16	43.43*
Calories calculated	26.32	38.87	40.30	42.53	40.47	40.64	44.15
Grams urinary N.....	0.272	0.865	1.724	1.80	1.88	1.99	1.93
No. of experiments....	(3)	(4)	(2)	(2)	(2)	(1)	(1)

*Dog awake part of the time.

It will be noticed that the metabolism rises from 25 calories in starvation, to 34 calories after the ingestion of 700 grams of meat, and from 25 calories to 40 calories, after the ingestion of 1,200 grams of meat. In general, the figures agree with Rubner's conception, that about 30 per cent. of the total energy of metabolized protein is wasted as free heat, within the organism. One new point stands out prominently in the above table, and that is, that for the first time, the direct and the indirect calorimetry in hourly periods have been found approximately to agree.

An exception to this occurs during the first two hours after the ingestion of 1,200 grams of meat. Here, the calories found are much smaller than the calories calculated by the Zuntz method of indirect calorimetry, that is from the carbonic acid excretion, the oxygen absorption and the nitrogen elimination. This indicates that the method of indirect calorimetry is not an accurate measure for the heat production during the early hours after the ingestion of meat in large quantity.

Two causes may here be active. Either (1) there is oxygen storage within the organism and carbonic acid elimination from the blood, or (2) there are oxidative processes which require oxygen and cause the liberation of carbon dioxide as the result of the production of early cleavage-products of protein metabolism, products which yield carbonic acid and require oxygen, but which do not liberate heat in the same quantities as it is liberated when the protein molecule is oxidized in its entirety.

36 (568)

The metabolism, directly determined, of healthy children during sleep.

By JOHN HOWLAND.

[*From the Department of Physiology, Cornell Medical College, New York.*]

Through the kindness of Dr. Lusk these experiments were made in conjunction with him, Dr. Williams and Mr. Riche.

As yet experiments have been made on only two children, but the results are given on account of the interest attaching to the first direct determination of heat produced by children.

The children were healthy, were gaining weight and were fed every four hours so as to keep their metabolism as constant as possible. They were put on the portable metabolism apparatus devised by Dr. Du Bois that allows great freedom, accurate collection of all the urine and feces and no discomfort. They were kept awake during the forenoon, fed at 1 p.m. and put at once into the calorimeter where they usually slept throughout the whole experiment.

Some of the results are given in the chart. It will be seen that the amount of heat produced varied considerably from hour to hour, and that during the same hours of different days with a constant diet the heat produced is not the same. The average for the hours in which the conditions were entirely satisfactory was 14.18 calories. The metabolism directly determined compared moderately with the metabolism as calculated in about half the cases. In the other half of the cases a low respiratory quotient indicated an erroneous oxygen determination, which invalidated the calculations.

Comparing the results in the two children it is found that the second, McG., regularly gave off more heat than the first (Newman) and that his heat production per square meter of surface per 24 hours was much greater. This is undoubtedly due to the greater surface area of the second child which fails to show by the ordinary formula for calculating the surface area ($12.3 \times \sqrt[3]{\text{weight}^2}$). Tne second child, McG. was 8 months old, weighing 4.320 kilos,

450 grams more than the first, Newman, who weighed 4.770 kilos, and he was 3½ cm. longer, but as the formula contains only one variable and that the weight, it gives a surface area for the second child less than for the first, though it is undoubtedly greater. The formula is apparently very accurate for well-nourished infants but not for the long and poorly nourished.

The heat directly determined and calculated for a square meter of body surface in twenty-four hours was in the two cases 994 and 1,093 calories. These figures correspond closely with the calculated heat in three of Rubner's cases, viz., 1,006, 1,143 and 1,090. The average CO_2 per square meter per hour was: Newman, 15.24; McG., 17.19.

HEAT PRODUCED BY INFANTS.

Newman (3 mos.)

1.45–2.45 P. M.		2.45–3.45 P. M.		3.45–4.45 P. M.	
Calculated.	Found.	Calculated.	Found.	Calculated.	Found.
17.17	17.01	15.96	13.62	15.86	14.91
		15.31	15.72	15.89	13.05
		18.16	13.85		
		13.06	13.42	17.47	13.48
		17.82	15.05	15.31	14.29
McG. (8 mos.)					
15.87	15.19	16.28	14.57	16.66	15.18
				18.02	15.64

37 (562)

Studies on human nephritis.

By **F. S. MEARA** and **A. I. RINGER.**

[*From the Department of Therapeutics of the Cornell University Medical College.*]

PRELIMINARY REPORT.

The object of this investigation was to determine the functional capacity of the kidneys of nephritics with regard to their power of eliminating nitrogenous material, water and salts, and to determine the influence of the "protective therapy" on the kidney efficiency.

We have up to the present the reports of nine cases that have been investigated for periods of two to ten weeks.

The patients after admission to the ward, except when an immediate change in diet was necessary, were placed on a general hospital diet for about three days. The urine and feces were collected and analyzed. After this preliminary test the patients were placed on a diet composed of two quarts of milk, with enough cream and sugar to supply the necessary calories. This diet contained 12 to 13 grms. of nitrogen, 3 grms. of NaCl and about 1,500 c.c. of water. A normal individual should reach nitrogenous equilibrium on about the third day of its administration. Several of our nephritic cases behaved in the following manner:

Case.	Duration of Period.	Average Daily N Intake "A."	Average Daily N Output in Urine "B."	B/A Per Cent.
E.B.	11 days.	12.5 grm.	8.89 grm.	71.1
W.C.	11 days	12.5 grm.	6.91 grm.	55.3

The relationship between the eliminated nitrogen to the ingested nitrogen, expressed in percentages we consider an index of the kidney's nitrogen eliminative power (N.E.P.) when on this standard diet. *A* minus *B* minus the amount of nitrogen found in the feces, represents the amount of nitrogen retained in the system due to the kidney inefficiency. That the nitrogen is not retained because of the building up of protein, but because of kidney inefficiency, is evident from the fact that N/SO_3, ratio in the urine of these cases is very low, and corresponds to the amount of nitrogen ingested.

Case M. with quite marked uræmic symptoms eliminated 4.51, 5.75 and 5.57 grms. of nitrogen per day, with 0.645, 1.113 and 0.97 grms. of inorganic SO_3 for the respective days. The N/inorg. SO_3 ratio during these days was 6.99, 5.17 and 5.72, *i. e.*, the patient eliminated about three times the amount of inorganic SO_3 that is ordinarily eliminated with the same amount of nitrogen.

The N.E.P. of our patients E.B. and W.C. was raised very considerably after several weeks of a "Protective" diet, *i. e.*, a diet containing less nitrogen than the N.E.P. as found in a preliminary test.

The mechanism for the removal by the kidney of one constitu-

ent of the urine may be impaired while the others remain perfectly intact. In case H. with an acute exacerbation of a chronic affection of the kidney due to an attack of tonsilitis, the NaCl eliminating power was absolutely lost. No precipitate could be obtained with $AgNO_3$ after the removal of the albumin, whereas on the same days the patient eliminated water, nitrogen and SO_3 perfectly well.

CASE H.

Date.	Volume of Urine.	Sp. Gr.	Total N.	NaCl	Inorg. SO_3.	$\frac{N}{\text{Inorg. } SO_3}$	Diet.
12/25/10	2405	1010	11.80	0.00	0.8021	14.7	General
12/26/10	2390	1011	11.32	0.00	1.0940	10.3	Salt free.
12/27/10	3120	1007	12.11	0.00	——	——	Salt free.

Similar results were also obtained in case Ba.

38 (563)

A note on the nature of oxyphilic granulation.

By **HERMAN M. ADLER.**

[*From the Danvers State Hospital, Hathorne, Mass.*]

An aqueous solution of eosin will give with ferric chloride a precipitate which has a deep red color. If very dilute solutions are used, say 0.1 of 1 per cent. ferric chloride, and an eosin solution which is just barely pink, no precipitate will be visible on mixing the two, but the pale pink of the eosin changes to a deeper shade of red, and the fluorescence, which is quite noticeable even at this dilution, disappears. If the dilution of the eosin solution used be increased so that these color changes can no longer be distinguished with certainty, it is possible to demonstrate the reaction by the addition of a colloidal suspension of Witte's peptone, or of sodium oleate.

On the addition of such a solution, which should be sufficiently strong to cause a well marked opalescence, the red color of the iron-eosin stain will at once be apparent, especially where sodium oleate has been used, in which case a flocculent precipitate stained a rich red will appear. The examination under the microscope of such a precipitate, reveals an appearance which, in respect to color

is apparently identical with that of oxyphilic granulation. It would appear that we have here not a true stain, in the sense of solution, but rather a case of condensation of the stain on the surface of colloidal particles.

The reaction thus detailed for eosin, is obtained also with Fuchsin S., though the eosin has a more marked color change, due to the loss of fluorescence. The experiment with the dilute solutions is comparable to the conditions under the microscope, where the thin layer of the specimen corresponds to the extreme dilution of the solution, and the visible staining of any tissue indicates a selected accumulation of such stain.

It has been shown that the granules of the eosinophile leucocytes contain iron, and it would seem that the reaction of these granules with eosin and fuchsin was due, in part at least, to this iron content.

The affinity of hæmoglobin for eosin is possibly dependent similarly upon its iron content.

Copper gives a similar reaction. The color change is, however, not quite the same, in that instead of a rich blood-red, the copper-eosin has a distinct purplish or bluish tinge.

Potassium bichromate gives the reaction though very weakly. This is suggestive, perhaps, of the effect of fixation by means of chromates.

In insufficiently stained blood smears, the granules of the eosinophiles, while characteristic in color, are superficially stained.

It is not probable that the stain depends upon a selective affinity, whereby an accumulation of dye is produced within the substance of the granule. It would appear, rather, that this reaction is of the physico-chemical sort, and occurs chiefly at the surface, between the more solid granule and the solution of stain.

39 (564)

Concerning the elimination of dextrose into the gastro-intestinal canal.

By **I. S. KLEINER.**

[*From the Department of Physiology and Pharmacology of the Rockefeller Institute.*]

Our experiments have reference to statements in literature, some of which, for a better understanding of our results, may be here quoted very briefly. On the basis of analyses of the contents of the gastro-intestinal canal of seven rabbits, Fischer and Moore make the statement that "the small intestine of the rabbit contains no sugar, when an animal is killed shortly after having consumed several hundred grams of carrots and carrot tops or cabbage." J. B. MacCallum reported that in a few experiments with intravenous injection of sodium chloride which, as is well known, produces glycosuria he found some dextrose in the stomach and intestines the quantity of which was slightly increased when, previous to the infusion of the sodium chloride, both kidneys were ligated. MacCallum thought that the presence of glucose in the intestines under these circumstances is due to the hyperglycemia and looked upon it as a sort of "intestinal diabetes"; he believed further that in the absence of the kidneys the intestines assume a supplementary excretory function. Fischer, on the other hand, draws the conclusions from some experiments that hyperglycemia alone does not lead to a secretion of glucose into the intestines. This occurs only, he maintains, when the infusion of sodium chloride is administered to hyperglycemic animals as was the case in MacCallum's experiments on account of the use of morphine.

In our experiments the rabbits had been fed for some time on hay and cabbage. The dextrose determinations were made by Pavy's method and controlled in some instances by Allihn determinations. Various series of experiments were carried out. Only the average figures of each series will be given here. In one series of six normal animals the contents of the stomach and intestines were analyzed some hours after they were fed. The small intestines as well as the stomach contained in each case small amounts of reducing substance. The averages

are: for the intestines 0.05 g. and for the stomach 0.1 g. In a second series six animals received by intravenous infusion 7 grams dextrose per kilo body-weight (in about molecular concentration). The average duration of the infusion was about half an hour; fifteen minutes later the animal was killed, the intestines were washed out and the stomach removed. The averages were: for the intestines 0.16 g. = 1.4 per cent. and for the stomach 0.13 g. = 1.1 per cent. of the injected dextrose. During the period between the beginning of the infusion until the animal was killed for the purpose of washing out the intestines an average of 39 per cent. of the injected dextrose was eliminated in the urine. In a third series of six rabbits only 4 grams of dextrose per kilo were injected (in about 0.6 molecular concentration). The average of this series amounted for the intestines to 0.11 g. = 1.9 per cent., and for the stomach 0.08 g. = 1.1 per cent. of the injected dextrose. Through the urine 23 per cent. of the injected dextrose was eliminated. In the fourth series of rabbits double nephrectomy was performed and on the following day 7 grams of dextrose per kilo were infused. The averages of this series are as follows: for the intestines 0. 24 g. = 2.1 per cent. and for the stomach 0.15 g. = 1.3 per cent. of the injectedd extrose. Finally in a series of only three nephrectomized rabbits the stomach and the intestines were examined on the following day without a preceding infusion of dextrose. Only a mere trace of dextrose could be discovered in these instances. These animals partook of very little food after nephrectomy.

Our experiments have shown that the stomach and especially the small intestines of rabbits contain measurable quantities of dextrose even when the food is poor in easily convertible carbohydrates; that an intravenous injection of dextrose increases perceptibly the gastro-intestinal dextrose even without the aid of a simultaneous infusion of sodium chloride, and that nephrectomy indeed increases the elimination of infused dextrose. However, considering the high elimination of the injected dextrose through the urine, the comparatively insignificant increase of elimination into the gastro-intestinal canal after nephrectomy can hardly be looked upon as a functional assumption of the activities of the kidney, as a vicarious phenomenon.

40 (565)

Experiments with chloroform, administered by intratracheal insufflation, in strychnine poisoning.

By **T. S. GITHENS** and **S. J. MELTZER.**

[*From the Department of Physiology and Pharmacology of the Rockefeller Institute for Medical Research.*]

Soon after the introduction of the method of intratracheal insufflation, experiments were begun to study the control of strychnine poisoning by means of this method. So far three series of investigations were carried out. In the first series Shaklee and Meltzer employed, besides insufflation, curare and intravenous infusions of Ringer's solution. In these experiments it was first established that in intravenous injections, 0.4 mgr. of strychnine represents a reliable fatal dose per kilo dog. When using insufflation, curare and Ringer's solution quite a large percentage of dogs were saved which received even more than the fatal dose. Out of 6 dogs which received 0.5 mgr. per kilo, 5 were saved, and out of 22 dogs which received 0.8 mgs. p. kilo 13 were saved.

In a second series of experiments, carried out by the present authors and communicated at the last meeting of the American Pharmacological Society, ether was substituted for curare. Twenty dogs which received 0.8 mgr. strychnine per kilo, that is, twice the fatal dose, were subsequently treated by intratracheal insufflation, ether and intravenous injections of Ringer's solution. All these dogs survived, none died later from after effects and when finally killed the autopsy revealed no abnormal conditions in any of the animals.

In medical practice chloroform is frequently employed in human cases of strychnine poisoning. We have therefore carried out a series of experiments in which chloroform was used instead of ether. We wish to present here the results of these experiments very briefly. Of 21 dogs which received intravenously 0.8 mgr. strychnine per kilo and were treated with chloroform and insufflation 12 survived and 9 died. The contrast to the series in which ether was used is striking. Furthermore, of these dogs only 11 received also infusion with Ringer's solution, 10 did not

receive such infusion. Of the 11 dogs which received Ringer's solution 6 died on the table and 2 died a few days later. Of the 10 animals which did not receive Ringer's solution, 7 survived, two died on the table and one died about 12 hours later. While when using ether the intravenous infusion was a definite favorable factor, it proved to be definitely unfavorable when chloroform was employed. Finally in nearly all the chloroform cases the autopsy revealed pathological conditions, either of the lungs or of the kidneys or of both. The acute deaths were brought about by pulmonary disorders accompanied mostly by pulmonary œdema.

When using the intratracheal insufflation there is no doubt that ether is a safer method than chloroform, at least in the treatment of strychnine poisoning.

41 (566)

A demonstration of osmotic pressure exerted by fat.

By **JACOB ROSENBLOOM** and **WILLIAM J. GIES.**

[*From the Laboratory of Biological Chemistry of Columbia University, at the College of Physicians and Surgeons, New York.*]

In the first of two demonstrations, the authors lowered a cylindrical *rubber* bag, one and one half inches in diameter and eight inches long, into an oiled *muslin* bag of about the same dimensions. The rubber bag was then filled to overflowing with olive oil. The rubber bag expanded, as the oil filled it, to the full length and width of the muslin sheath. The sheath prevented further extension of the rubber bag and imparted rigidity to the osmometer that was ultimately constructed. The full double bag, with its mouth wide open, was then raised so as to enclose about an inch of the lower end of a long glass tube which was firmly supported vertically above the demonstration table. The glass tube was 5 feet long and its bore was 4 mm. in diameter. Ligatures were tightly secured around the neck of the double bag against the immersed lower end of the vertical tube. The bag then hung directly from the end of the tube. The bag and its sheath were in a tightly distended condition and a station-

ary column of oil an inch high in the tube was visible above the protruding edge of the sheath. The tube and bag were then lowered into a salt mouth liter bottle on the table until the bag almost touched the bottom of the bottle. The height of the bottle and the length of the bag were nearly equal. The tube was then marked with a label on the plane of the oil meniscus just above the neck of the bag, and enough ether was poured into the bottle to provide immersion for the bag to the depth of an inch. For a moment no change in the volume of oil was apparent, and the lateral pressure of the ether was obviously without mechanical effect. But in a minute or two diffusion currents were visible along the surface of the bag and oil rose rapidly in the tube.

After the initial effects of the ether had been shown, the bottle was filled with ether containing Sudan III, and a 5-foot vertical extension of the same bore was added to the upright glass tube. In a moment the upward movement of the liquid was accelerated. The demonstration was started at about 9 p.m. At 10 p.m. the osmotic pressure had carried the column of oily fluid to the top of the 10-foot tube, and liquid continued to run rapidly from the upper orifice until the apparatus was dismantled after the adjournment of the meeting, at about 11:30 p.m.

During the progress of the demonstration, Sudan III diffused rapidly from the exterior, through the rubber, to the very top of the rising column of fluid, before any of the liquid passed out of the upper opening. Oil diffused rapidly through the rubber into the ether.

The second demonstration was essentially the same in principle and technic as the first. Instead of a 10-foot upright tube, however, the authors substituted an L tube with an inside diameter of 6 mm. The vertical extension of the tube was 17 in., the horizontal extension was only 3 inches. The latter extension was drawn out to a narrow bore in an inclined plane, to facilitate direct delivery of any liquid that might pass through that end of the tube.

When partial immersion of the bag first occurred there was no visible response, but, in a minute or two, oil began to rise in the tube. The bag was then completely covered with ether. The upward movement proceeded rapidly and in about an hour nearly

200 c.c. of liquid passed through the upper orifice into a graduated cylinder which was supported underneath it to catch the overflow.

The authors are engaged in a study of various relationships that are suggested by the demonstrated phenomena.

43 (568)

A demonstration of the diffusion of pigments from fat through rubber into fat.

By **WILLIAM J. GIES.**

[*From the Laboratory of Biological Chemistry of Columbia University, at the College of Physicians and Surgeons, New York.*]

The author has found that many fat-soluble pigments, such as Sudan III and Scarlet R, readily diffuse from solid and liquid fats through rubber into various solid and liquid media, among them both solid fat and oil. Thus, when Sudan III is dissolved in melted lard, the red liquid poured into a rubber bag, the bag supported in melted lard in a bottle, and the apparatus promptly immersed in ice water—the fatty matter will congeal before any sign of pigmentary diffusion occurs, but, in a few hours, a reddish tinge will develop outside of the bag, and on each successive day for several weeks further extension of the pigmented matter may be witnessed, until the whole of the external lard is deeply suffused with red. This process takes place quite rapidly when the lard and apparatus are kept in a thermostat at 40° C.

The demonstrations were intended to exhibit a few instances of such pigmentary diffusions as occur speedily enough at room temperature to yield positive results within an hour. The appended

	Contents of the Rubber Bag.		Nature of the Liquid in which the Bag was Suspended.	Result.
	Oil.	Pigment.		
1	Olive oil.	Scarlet R.	Olive oil	Visible diffusion of the pigment occurred promptly.
2	Cocoanut oil.	Scarlet R.	Cocoanut oil.	Visible diffusion of the pigment occurred promptly.
3	Lard oil.	Sudan III.	Lard oil.	Visible diffusion of the pigment occurred promptly.
4	Paraffin oil.	Sudan III.	Paraffin oil.	Visible diffusion of the pigment occurred promptly.
5	Olive oil.	Sudan III.	Ether.	Visible diffusion of the pigment occurred *almost immediately.*

summary indicates briefly the precise nature and results of the demonstrations (including two control tests—4 and 5), which were made with thin rubber bags in ordinary glass bottles.

The bags were securely supported in the bottles and the mixtures were shaken occasionally during the demonstration. The bags were found, after the adjournment of the meeting, to be without defects.

Numerous related experiments are now in progress.

42 (567)

Comparative dialysis experiments, with demonstrations.[1]

By **F. G. GOODRIDGE** and **WILLIAM J. GIES.**

[*From the Laboratory of Biological Chemistry of Columbia University, at the College of Physicians and Surgeons, New York.*]

When dry bags of rubber, gold beaters' skin, parchment and collodion, each containing olive oil and Sudan III, are separately immersed in olive oil, and the remaining conditions of the environment are uniform, diffusion of the pigment promptly occurs through rubber, but does not take place at all through any of the other three membranes. When the bags are lifted from the oil, washed externally with ether, and then immersed in ether,[2] the pigment quickly passes through the rubber, but diffuses very slowly if at all through the remaining membranes.

Successive immersions of *moist* impermeable membranes (gold beaters' skin and parchment) in alcohol and ether, for different periods of time, fail to render the treated membranes more per-

[1] This and the two preceding communications relate to studies in a projected series on *physico-chemical conditions in the cell*, which in turn constitutes a section of a comprehensive plan of research on the composition of protoplasm as well as the structural and dynamic relationships of cell constituents and products. These investigations are now in progress in the Laboratory of Biological Chemistry of Columbia University, at the College of Physicians and Surgeons, and under the auspices of the George Crocker Special Research Fund.

[2] In experiments which the senior author has been conducting with Dr. Welker's cooperation, it has been found that collodion bags are disintegrated by *ether containing more than about 1.5 per cent. of alcohol. Pure ether* does not dissolve or in any way disorganize collodion membranes. A collodion bag containing *pure* ether may be immersed for a week or more in *pure* ether without undergoing any appreciable deterioration.

meable to Sudan III under the conditions of the experiments already described.

The authors demonstrated the general facts in this connection pertaining to rubber and gold beaters' skin.

Experiments along these lines, with additional membranes, pigments and liquid media, are in progress, in an effort to obtain further knowledge of the functions of membranes in diffusion.

44 (569)

Occurrence of spontaneous lesions in kidneys and livers of rabbits and guinea pigs.

By **W. OPHÜLS.**

[*From the Pathological Laboratory of Cooper Medical College, San Francisco.*]

In view of the importance of the occurrence of spontaneous lesions in the kidneys and in the livers of rabbits and of guinea pigs in reference to the experimental work on these organs, Dr. E. C. Dickson and myself have made a careful study of these organs and incidentally of the heart and of the aorta in fifty rabbits and in one hundred guinea pigs. The animals used were partly fresh animals from the market, partly animals raised at the laboratory.

Many of the rabbits had been used in the physiological laboratory and had been killed immediately after the experiments; some died of coccidiosis; a few had been injected with material supposedly containing pneumococci without result; others had never been used. Several old rabbits that had been in the laboratory for a year or longer were especially selected on account of the greater likelihood of the existence of renal or hepatic lesions in them. Twenty-eight rabbits, among them some of the old ones, had entirely normal kidneys, nine showed slight parenchymatous lesions, three a few small areas of cellular infiltration. In ten we found scattered small areas in which were marked interstitial lesions with the formation of small depressions on the surface. Four of these proved to be radially arranged chronic septic foci which extended from the vicinity of the papilla to the outer surface.

In the other six cases the same arrangement of the new formed connective tissue in narrow radial bands which start deep in the pyramids was noticeable although no evidence of a septic infection could be discovered. The interstitial lesions were accompanied by marked epithelial lesions in two cases only, producing to a certain extent the picture of a chronic parenchymatous nephritis, although the lesions were never very extensive. Lesions of the blood-vessels or primary lesions of the glomeruli were not found in any case. The lesions described by the writer in his paper on experimental chronic nephritis[1] in rabbits 52 and 48 are probably spontaneous ones. Only six normal livers were encountered; the rest showed various stages of coccidial infection with more or less cirrhosis and sometimes cirrhotic processes in cases in which coccidia could not be demonstrated. We are very strongly of the opinion that our findings throw very serious doubt on the numerous reports of the experimental production of cirrhosis in the liver of rabbits as this animal would seem to be entirely unsuitable for such experiments. No gross lesions of the heart or of the aorta were discovered in any of the animals.

Of the one hundred guinea pigs the large majority had been injected with sediment from urine or other material suspected of containing tubercle bacilli; a few only actually developed tuberculosis. There were several especially selected old animals. Sixty-three of the one hundred had absolutely normal kidneys, two showed slight parenchymatous lesions and in thirty-five there were scattered small areas of cellular infiltration in the cortex which were sometimes rather numerous, at other times they were found with difficulty only on careful examination of several sections. They consist largely of lymphocytic cells and few larger cells with vesicular nuclei. They do not seem to have much tendency to develop into fibrous tissue although occasionally such a change was observed but always to a limited degree only. A reason for the existence of these foci could not be discovered. The lesions described in the writer's article on "Experimental Chronic Nephritis"[2] in guinea pigs as representing the early stages of renal lesions in chronic lead poisoning are probably of

[1] *Journal of Medical Research*, 1908, XVIII., 497.

[2] *Journal American Medical Association*, 1907, XLVIII., 483.

this character. In few cases fibrous thickening of the capsules of glomeruli and cystic dilatation of the same were observed as spontaneous lesions similar to certain types of glomerular cysts described by E. C. Dickson in his paper on the "Experimental Production of Chronic Nephritis in Animals by the Use of Uranium Nitrate."[1] These were, however, rare exceptions, whereas in uranium poisoning such glomerular lesions seem to be nearly constant. Large scars or lesions of the blood vessels were not found in any case.

Of the livers two showed extensive necroses, a disease probably familiar to all who handle many guinea pigs. In one case there was a slight cellular infiltration of the periportal connective tissue and in two sufficient new formation of connective tissue to speak of it as an incipient cirrhosis. A condition resembling cirrhosis therefore seems to occur spontaneously in guinea pigs also, but, so far as our observations go, it is quite rare. Heart and aorta were carefully inspected in all cases, but nothing abnormal was noted in any instance.

45 (570)

Spontaneous nephritis in wild rats.

By **W. OPHÜLS.**

Among the very many rats examined in San Francisco for plague only very few were found that showed evidences of dropsy and of renal disease. One such rat was carefully examined at our laboratory through the courtesy of the U. S. Public Health and Marine Hospital Service. This rat showed a very marked general oedema. The kidneys are small, distinctly granular. The measurements are 26×14×10 mm. The heart is moderately but distinctly enlarged, measuring in the formalin hardened specimen from base to apex—23 mm., the largest transverse diameter is 19 mm. and the largest antero-posterior diameter 14 mm. There are no gross lesions of the aorta. In sections of the kidneys the blood-vessels are found in a normal condition. The glomeruli are also quite normal except a few which show slight fibrous thickening

[1] *Archives of Internal Medicine*, 1909, III., 375.

of capsule and slight cystic dilatation. The epithelium in practically all tubules is very markedly and extensively degenerated, showing granular and fatty degeneration. There are many casts. Large bunches of peculiar unidentified, uncolored needle-shaped crystals are also found in the tubules. Large areas in the sections show collapse of tubules with much cellular infiltration and new formation of cellular fibrous tissue between them. The appearances are those of a chronic parenchymatous nephritis. It is possible that these lesions are the result of one or the other poison, such as arsenic or phosphorus, which were used in destroying rats in this city. In this connection it is noteworthy however how rarely such lesions were observed in spite of the very extensive use of such poisons.

46 (571)

The stimulation of adrenal secretion by emotional excitement.

By **W. B. CANNON** and **D. DE LA PAZ.**

[*From the Laboratory of Physiology in the Harvard Medical School.*]

Dreyer's demonstration that splanchnic stimulation increased the content of adrenal secretion in blood from the adrenal veins has been confirmed by several observers. Adrenal secretion therefore is under control of the sympathetic system.

Major emotional disturbances indicate the dominance of sympathetic impulses. In the cat, for example, fright causes dilation of the pupils, inhibition of the stomach and intestines, rapid heart, and erection of the hairs of the back and tail. Do not the adrenal glands share in this widespread subjugation of the viscera to sympathetic control?

To try this suggestion the inhibition of contraction in strips of longitudinal intestinal muscle, sensitive to suprarenin 1:20,000,000, was used as a biological test. Blood was obtained from the cat when quiet, and again after the animal was excited by the presence of a barking dog, by introducing, through the femoral vein, into the inferior vena cava to the region of the liver, a small vaselined catheter. The blood thus obtained was defibrinated and applied to the intestinal strip at body temperature.

After an initial shortening the strip contracted rhythmically in blood from a quiet animal. In no instance did such blood produce inhibition. On the other hand, blood taken from animals after the emotional disturbance, showed more or less promptly the typical relaxing effect. As the emotional period was prolonged, the effect became prompter and more profound.

The view that inhibition of the contracting intestinal strip is due to an increased content of adrenal secretion is justified for the following reasons. (1) The effect was obtained in blood from the vena cava near the liver when that from the femoral vein taken simultaneously produced no inhibition. (2) Removal of the adrenal glands after tying the adrenal vessels resulted in a failure of excitement to produce the effect. (3) Adding varying amounts of adrenalin to inactive blood evoked all the degrees of relaxation that have been observed in excited blood. (4) Excited blood which produced prompt inhibition lost that power on standing or on being agitated by bubbling oxygen. These conditions together with the evidence that sympathetic impulses increase the secretion of the adrenal glands, and that during such emotional excitement as was here employed signs of sympathetic discharge were observable in the animal from the eye to the tip of the tail, prove that the effect was due to adrenal secretion.

Injected adrenalin is capable of inducing an atheromatous condition of the arterial wall in rabbits, especially in elderly individuals, and is also capable of evoking hyperglycemia with glycosuria. As Ascher has shown, by prolonged stimulation of the splanchnic nerves, prolonged secretion of the adrenal glands with maintained high blood pressure can be produced. In the light of the results here reported the temptation is strong to suggest that some phases of these pathological states are associated with the strenuous and exciting character of modern life acting through the adrenal glands. This suggestion, however, must be put to experimental test.

47 (572)

The production of glycosuria as a result of the intravenous injection of Witte's peptone.

By **YANDELL HENDERSON** and **FRANK P. UNDERHILL.**

[*From the Physiological Laboratory, Yale Medical School and the Sheffield Laboratory of Physiological Chemistry, Yale University.*]

Renewed investigation concerning the phenomena provoked by intravenous administration of Witte's peptone has demonstrated the production of a marked glycosuria, following such injections. The appearance of sugar in the urine is accompanied by hyperglycæmia. The experiments were carried out for the most part upon dogs. When Witte's peptone is injected into the rabbit glycosuria is not in evidence, an observation which is in entire accord with the failure of this substance to induce certain other phenomena in this animal which are brought about in the dog.

The tentative hypothesis is advanced that the presence of sugar in the urine is induced as a result of the respiratory disturbances set up by the "peptone" injection.

The phenomena connected with the injection of "peptone" mixtures are being subjected to further investigation.

48 (573)

Tubercle bacilli in the feces of cattle.

By **W. J. MACNEAL** and **C. F. BRISCOE.**

[*From the Agricultural Experiment Station, University of Illinois, Urbana, Ill.*]

Between June, 1908, and August, 1910, a number of tests upon the feces of cattle were made at the Illinois Agricultural Experiment Station to determine the presence or absence of tubercle bacilli. These tests were of two kinds, (1) by direct microscopic examination and (2) by inoculation of animals.

The microscopic method included the examination of stained slide preparations of (*a*) mucus on the exterior of the fecal mass,

(*b*) the mixed feces, and (*c*) watery mucus scraped from the rectum, each stained slide being searched fifteen minutes with the aid of a mechanical stage. If organisms indistinguishable in appearance from bovine tubercle bacilli were found upon any of these slides the examination was recorded as positive. One hundred and nineteen samples from 53 cows which had reacted to tuberculin were examined in this way, with a positive result 53 times in samples from 34 of the cows. Thirty-five other samples from these same cows were negative. Nineteen cows gave negative results at all examinations, 31 in number. Thus, of the 53 cows tested, 34, or 64.2 per cent., gave a positive result at one or more tests, and 19, or 35.8 per cent., gave only negative results. Forty samples from 18 non-reacting cows were tested in the same way, with 23 positive and 17 negative results. Fifteen of the 18 cows, or 83 per cent., gave a positive result at one or more tests, while only 3, or 17 per cent., were negative at all tests. From this it appears that no significance in respect to the presence of tubercle bacilli can be attached to the finding of a few acid-fast bacteria in the feces of cattle.

In order to test the delicacy of guinea pig inoculation as a test for tubercle bacilli in the feces of cattle, a number of experiments were performed in which accurately measured minute quantities of a pure culture of bovine tubercle bacilli were added to definite amounts of cow feces, previously shown to be free from tubercle bacilli. In this way it has been ascertained that as small a quantity as 1×10^{-11} gram of tubercle bacilli, suspended in one cubic centimeter of emulsion of feces, produces generalized tuberculosis upon subcutaneous injections into guinea pigs. The actual number of bacterial cells in this amount of culture would probably be between 5 and 500.

One hundred and four samples of feces from 62 reacting cattle have been tested by inoculation of guinea pigs. Virulent tubercle bacilli were thus detected in feces from three of these animals, or practically 5 per cent. No relation was found between the presence of visible acid-fast bacteria in the microscopic test and the presence of virulent tubercle bacilli as shown by guinea pig inoculation.

Whether the percentage of reacting cattle passing tubercle

bacilli in the feces is relatively large or small would appear to depend upon the stage of the disease in the individual animals. It is important to point out, that even in well-nourished and apparently healthy herds of reacting animals (such as those used in this work) there are likely to be, at all times, a few animals actually passing virulent tubercle bacilli in the feces.

A detailed account of this work is published in Bulletin No. 149 of the Illinois Agricultural Experimental Station.

49 (574)

An alteration of the sex-ratio induced by hybridization.

By **T. H. MORGAN**

[*From the Department of Zoölogy, Columbia University.*]

Males and females of the fly *Drosophila amphelophila* occur in nature in about equal numbers. A long series of experiments failed to induce any change in the sex-ratio by adding different sugars, salts, acids or alkalies to the food on which the larvæ live. A remarkable alteration of the sex-ratio took place, however, in the second generation of certain crosses between two races that arose as mutations. When a male of a race with rudimentary ("short truncated") wings was crossed to a female of a race with short proportionate wings all of the females had normal wings and all of the males short proportionate wings, like the mother. When these (F_1) were inbred, there were produced in the second generation three classes of individuals according to the character of the wings; namely, long (normal), short proportionate, and short rudimentary distributed according to sex as shown below:

Long ♀	Long ♂	Propt ♀	Propt ♂	Rudim ♀	Rudim ♂
989	137	336	389	8	12

The normal males are to the females as one to seven, while in the other two classes the sex ratio is approximately normal. *The point of prime importance is that it is the normal males that are affected.* Even if we assumed that the deficiency might be due to the absence (in part) of males with rudimentary wings, and double the number of males with normal wings, the number of normal males would still fall far below that of the females.

The reciprocal cross, namely, short proportionate males and rudimentary winged females gave, in the first generation, long-winged males and females. These inbred produced in the second generation:

Long ♀	Long ♂	Propt ♀	Propt ♂	Rudim ♀	Rudim ♂
265	80	36	90	0	14

Again, the sex disproportion is observed for the long-winged flies, while the reverse order holds now for the proportionate wings.

The F_2 flies from both of the above sets have been bred again in all possible combinations within the same set, and the disproportion of males to females was found to be transmitted to the next generation, although not in the same ratios. Some of the proportionate winged males (F_2) were also bred to wild flies, and gave 301 females and 111 males.

The most probable explanation of these results would seem to be either in the partial degeneration of the male producing spermatozoa as a result of crossing with the race with rudimentary wings or in some quantitative change in the sex determining factor. The effects are transmitted also to a third generation. Whether they are permanent or not remains to be tested.

SCIENTIFIC PROCEEDINGS.

ABSTRACTS OF THE COMMUNICATIONS.

Forty third meeting.

The Laboratory for Hygiene, University of Pennsylvania. April 19, 1911. President Morgan in the chair.

50 (575)

On the regular seasonal changes in the relative weight of the central nervous system of the leopard frog (R. pipiens).

By **HENRY H. DONALDSON.**

[*From the Wistar Institute of Anatomy and Biology.*]

The relative weight of the central nervous system of the frog, *Rana pipiens*, changes during the active season, and such a change is probably characteristic for other species of frogs with like habits.

The relative weight of the central nervous system is low at the time of emergence, high in the midsummer (July) and low again at the time of hibernation. During hibernation it remains nearly constant. In the formula[1] used to express the weight of the central nervous system, the absolute value of C is characteristic for the station from which the frogs come.

The range from minimum to maximum in the value of C is about 13 per cent., rising 7 per cent. from the end of March to the end of April, 4 per cent. more from the end of April to the end of May, and 2 per cent. more from the end of May to the first of July, remaining stationary in July and then falling month by month at a similar rate to the end of October.

This variation in the relative weight according to season is due to lack of coincidence between the growth of the central nervous system and the growth of the entire body.

[1] This formula is as follows: Weight of C. N. S. = (log. $W.\sqrt[4]{L.}$) C indicating that the weight of the central nervous system is equal to the log. $W.$, body weight in grams, by $\sqrt[4]{}$ of the total length L, in mm., this product to be multiplied by C, a constant.

In frogs from one to four years old, the body weight more than doubles during each active season, although the precise form of the curve representing this body growth is not known.

The growth of the central nervous system is precocious in relation to that of the body, but in the absence of direct observations on the growth of the body, the form of the curve can only be indirectly determined.

During the active season, the percentage of water in the entire frog falls slightly from spring to summer and rises again from summer to autumn. These changes seem to be due to the combined effects of advancing age and varying food supply.

51 (576)

An interpretation of growth curves from a dynamical standpoint.

By **S. HATAI.**

[*From the Wistar Institute of Anatomy and Biology.*]

The growth phenomena may be considered as a gradual transformation of growth energy to the work done in forming the mass which composes the body. The present writer wishes to determine "what law, if there is any, governs in any individual the rate of transforming the growth energy into the work done."

In order to solve the above problem, the assumption was made that under normal conditions the growth energy is transformed into the work with least loss of energy. It was shown that in order that this assumption should be true we must have $\delta A = 0$ in the following integral

$$A \text{ (action)} = \int mvds.$$

Applying this principle it was proved that the formula for the growth of brain in weight (and any other data which satisfy the same conditions) must be a function which renders the following integral minimum

$$u = k\int\left(\frac{1}{x}+\frac{1}{a}\right)\sqrt{1+\left(\frac{dy}{dx}\right)^2}\,dx.$$

The integral is minimized when the function y has the following relations:

$$y = \frac{ca}{k\left(1 - \frac{c^2a^2}{k^2}\right)^{\frac{1}{2}}} \sqrt{\left(x + \frac{a}{1 - \frac{c^2a^2}{k^2}}\right)^2 - \frac{c^2a^4}{k^2\left(1 - \frac{c^2a^2}{k^2}\right)^2}} - \frac{ca^2}{k\left(1 - \frac{c^2a^2}{k^2}\right)^{\frac{3}{2}}}$$

$$\times \log \left\{ \left(x + \frac{a}{1 - \frac{c^2a^2}{k^2}}\right) + \sqrt{\left(x + \frac{a}{1 - \frac{c^2a^2}{k^2}}\right)^2 - \frac{c^2a^4}{k^2\left(1 - \frac{c^2a^2}{k^2}\right)^2}} \right\} + c_2.$$

If our assumptions are correct, the above formula ought to represent adequately our growth curves. It was shown that the above formula can be transformed into the following forms as particular cases:

$$y = a + b \log (x + c),$$
$$\text{and } y = a + bx + c \log x.$$

These are formulas which are already extensively used for graduating observed growth curves. Thus there is no further need to test the adequacy of the formula to represent the growth curves, since numerous applications have been made with satisfaction and already published by various investigators. I therefore put forward the following provisional definition of growth considered as a process: "An organism tends during growth to form greatest amount of mass with least loss of growth capacity."

Further the present investigation furnishes a biological meaning to the logarithmic formulas which have been extensively used without appreciating the full significance of their properties.

The cases of abnormal growth were also discussed but will be treated more fully in a future publication.

52 (577)

Experiments to modify the sex ratio in the toad.

By **HELEN DEAN KING.**

[*From the Wistar Institute of Anatomy and Biology.*]

Several series of experiments were made in the spring of 1910 in order to ascertain whether the sex ratio in the toad can be altered by subjecting the eggs to different environmental conditions at or before the fertilization period.

Lots of eggs fertilized in various solutions of alcohol (.13 per cent. to 2 per cent.), as well as those fertilized with sperm from

the right or from the left testicle of three different males, all gave practically normal sex ratios as the proportion of females ranged from 46.92 per cent. to 55.64 per cent.

Three batches of eggs from two different females were fertilized out of water and kept in a moist chamber for several hours. Each batch of eggs gave an unusual excess of females, the proportion of females varying from 60.86 to 70.83 per cent.

Mature eggs from another female were subjected to the action of a 2.5 per cent. solution of salt or of cane sugar for ten minutes and then fertilized in tap water. In each case 70 per cent. of females was obtained. In this series of experiments, as well as in the preceding one, it is probable that the eggs lost water during the fertilization period or at least were prevented from absorbing water during this time.

Seven lots of eggs from four different females were fertilized in solutions of hydrochloric or of acetic acid, the strength of the solutions varying from .01 per cent. to .0025 per cent. In every instance the percentage of females obtained was from 10 to 20 per cent. lower than that which is probably normal for the species. Lots of eggs from the same females fertilized in alkaline solutions (NH_4OH or NaOH) of the same strength gave proportions of the sexes within the range of probable normal variations in the sex ratios of different lots of individuals. It is probable that the acid solutions caused the eggs to absorb an extra amount of water during the fertilization period. The alkaline solutions were apparently too weak to have any influence on the eggs.

No definite conclusions can be drawn from these experiments, since in every case the mortality was very great. The results strongly suggest, however, that in the toad, *Bufo lentiginosus*, sex is determined at or near the time of fertilization and that it can be influenced by external factors. They also seem to indicate that the relative amount of water in the egg at the time of fertilization has some influence in determining sex: an increase in the water content tending to produce a male; a lowering of the water content favoring the development of a female.

53 (578)

Evidence that the primary change in stimulation is an increase in the permeability of the limiting membranes of the irritable elements.

By **RALPH S. LILLIE.**

[*From the Marine Biological Laboratory, Woods Hole, and the Physiological Laboratory, Zoölogical Department, University of Pennsylvania.*]

Various facts and theoretical considerations indicate clearly that the process of stimulation in muscle or nerve has its seat at the semi-permeable boundary layers or plasma membranes of the irritable elements, and consists in a sudden and reversible increase in the permeability of these membranes. After a brief review of this general evidence the following experiments were described.

I. *Experiments with the Larvæ of Arenicola cristata.*—These are the free-swimming ciliated larvæ of a marine annelid; they are small worm-like organisms about 0.3 mm. in length, readily obtained in large quantity by rearing. The larvæ have a well-developed muscular system; the special peculiarity which fits them for the purpose of the following experiments is the presence throughout the whole body of a water-soluble yellow pigment; this substance is contained *within the cells*, and does not visibly leave the latter except under conditions of markedly increased permeability—as on death or after treatment with cytolytic substances (*e. g.*, saponin); it then diffuses into the medium and, if sufficient larvæ are present, colors the latter a bright straw yellow. Its exit thus serves as a convenient index of increased permeability. It was found that during strong chemical stimulation a rapid loss of pigment always occurs: the rate and degree of this loss run closely parallel with the intensity of the stimulating action, as indicated by the extent and duration of the muscular shortening. Pure isotonic ($m/2$) solutions of neutral sodium salts ($NaCOOCH_3$, NaCl, NaBr, $NaNO_3$, $NaClO_3$, NaI, NaCNS) all cause strong and persistent muscular contraction accompanied by rapid loss of pigment; the addition of a little calcium chloride to the solution (1 c.c. $m/2$ $CaCl_2$ to 25 c.c. $m/2$ sodium salt) prevents both the

stimulating action and the loss of pigment. Potassium salts show a similar stimulating and permeability-increasing action, neither of which, however, is checked by the addition of calcium. Isotonic LiCl solution shows moderate stimulation with moderate loss of pigment; both are checked—as in the case of NaCl—by calcium. On the other hand while pure *m*/2 CsCl produces well-marked stimulation and loss of pigment, the addition of calcium does not check, but on the contrary markedly accentuates both effects. Mixtures of potassium and magnesium chlorides show varying action according to the relative proportions of the salts; in pure isotonic $MgCl_2$ solution there is neither stimulation nor loss of pigment, but complete reversible muscular anæsthesia; the same is true of mixtures containing a decided excess of $MgCl_2$ (*e. g.*, 1 volume *m*/2 KCl+4 volumes *m*/2 $MgCl_2$); when the proportion of KCl is increased to one half or more, stimulation and with it loss of pigment appear; in mixtures of equal parts both effects are slight; in mixtures of 2 vols. *m*/2 KCl to 1 vol. *m*/2 $MgCl_2$ both are somewhat increased; in a mixture of 4 vols. *m*/2 KCl to 1 vol. *m*/2 $MgCl_2$ there is moderate stimulation with moderate loss of pigment, though both effects are decidedly less marked than in pure *m*/2 KCl. Saturated solutions of chloroform or ether in sea-water produce strong contraction with rapid loss of pigment; weak solutions anæsthetize without stimulating or visibly increasing permeability.

II. *Experiments with Frog's Muscle.*—It was pointed out that many cytolytic substances produce slow and usually irreversible contraction in vertebrate skeletal muscle; this is the case with soaps, bile-salts, various hæmolytic alkaloids or glucosides (*e. g.*, saponin, digitalin, solanin, agaricin), strong solutions of lipoid solvents (chloroform, ether, benzol, toluol, etc.), certain foreign blood sera and certain bacterial toxins (*e. g.*, tetanus). The contraction is typically slow and steady, unaccompanied by twitching, and passes over into permanent rigor. It was found that after treatment of the muscle for some minutes with pure isotonic solutions of various neutral sodium salts the response to many of the above substances is so altered that rapid and vigorous contractions with twitching may result. This sensitizing action increases in the order: NaCl, NaBr, $NaNO_3$, $NaClO_3$, NaCNS and NaI, being usually slight

with the first two salts, and well marked with the others, particularly with iodide and sulphocyanate. Frog's gastrocnemii immersed for five minutes in *m*/8 NaI or *m*/8 NaCNS show rapid contraction and twitching when immersed in isotonic solutions of the following substances in Ringer's solution or physiological salt-solution: saponin, digitalin and solanin (marked action); agaricin and aconitine (relatively slight action); chloroform (marked action); Na-oleate (marked action); bile-salts (marked action); horse and dog serum (marked action with vigorous twitching); tetanus toxine (vigorous twitching); rattlesnake venom (moderate action). The intensity of the stimulating action shows a general parallelism with that of the hæmolytic action.

Muscles may be similarly sensitized to osmotic stimuli (distilled water and hypertonic sodium chloride solution). This fact, as well as the fact that colloidal substances (serum, etc.) may show marked stimulating action, furnishes additional proof that stimulation depends essentially on an alteration of the plasma membrane.

54 (579)

Nature of the muscular contraction.

By **E. B. MEIGS.**

[*From the Wistar Institute of Anatomy and Biology.*]

The comparison of histological preparations of uncontracted and contracted smooth muscle indicates that during the contraction of this tissue fluid passes from the fibers to the interstitial spaces. It seems possible, therefore, that the contraction of smooth muscle may be brought about by an interchange of fluid between its cells and their surroundings in the same way that the movements of *Mimosa* are caused by changes in the turgor of its cells. This hypothesis may be tested by investigating the effect of swelling reagents and their opposites on the length of smooth muscle. The hypothesis would be supported if it could be shown that smooth muscle lengthened when immersed in solutions which cause it to gain in weight and shortened in the opposite class of solutions.

The changes of weight and the changes of length of frog's smooth muscle have been followed in Ringer's solution, in various

modifications of Ringer's solution, in 0.7 per cent. NaCl solution, in isotonic saccharose, glucose, glycerine and alanin solutions. In all the cases investigated it has been found that increase in weight goes hand in hand with increase in length and that decrease in weight is accompanied by decrease in length. The changes in weight and the changes in length are roughly proportional to each other in rate and amount.

A suggestion of the nature of the chemical change which brings about contraction is found in the following facts. Smooth muscle usually gains in weight and lengthens in Ringer's solution which has the following formula: NaCl, 0.65 gram; KCl, 0.02 gram; $CaCl_2$, 0.025 gram; $NaHCO_3$, 0.02 gram; H_2O, 100 c.c. If for the $NaHCO_3$, 0.01 gram of lactic acid be substituted, the gain in weight and lengthening do not occur; there may even be a small loss in weight and shortening. Larger amounts of lactic acid may cause gain in weight and lengthening. If the muscle be stimulated with a strong interrupted Faradic current after it has been for some time in the weakly acid Ringer, it responds to the stimulation by lengthening slightly instead of by shortening. These facts may be explained by supposing that smooth muscle responds to stimulation like striated muscle by the production of a small amount of lactic acid. Under ordinary circumstances this production of lactic acid causes the fibers to lose fluid and shorten; but if the fibers have been artificially acidified before the stimulation takes place, the production of more acid on stimulation causes the fibers to take up fluid and lengthen.

55 (580)

Preliminary note on the action of some internal secretions upon erectile tissue.

By **ISAAC OTT** and **JOHN C. SCOTT.**

[*From the Medico-Chirurgical College of Philadelphia.*]

To study the action upon erectile tissue we employed the penile organ of the dog. The length of it was measured by a pair of compasses, from the bulb to the tip. The width of the bulb of the organ was measured in the same way. Then the filtered infusion made with distilled water was injected into a vein and the

dimension of the organ measured as before. It was found that the prostate increased the length 25 mm., and the width of the bulb 25 mm. The orchitic extracts gave an increase in length of 15 mm., and an increase in width of bulb of 23 mm. The ovarian extract gave an increase in length of 21 and an increase in width of bulb of 23 mm. The parathyroid extract gave an increase in-length of 15 and an increase in width of bulb of 15 mm. The thymus extract gave an increase in length of 15 mm. and an in crease in width of bulb of 12 mm. Pituitary extract as a whole, gave an increase in length of 11 mm. and an increase in width of bulb of 6 mm. Infundibulin gave an increase in length of 5 mm. and an increase in width of bulb of 6 mm. Pineal gland gave no increase in length but increase in width of bulb of 7 mm. The corpus luteum increased the length 15 mm. and the width of the bulb 28 mm.

56 (581)

The depressor action of dog's pancreas and pancreatic juice.

By **A. B. EISENBREY** and **R. M. PEARCE.**

[*From the Laboratory of Research Medicine, University of Pennsylvania.*]

The marked lowering of blood pressure produced by the pancreatic juice of the dog when injected into the circulation of the dog, suggested that the sudden onset of prostration and collapse, symptoms indicative of vasomotor insufficiency seen clinically in acute pancreatic disease, might be due to the direct peritoneal absorption of the pancreatic juice set free in the destruction of the gland.

We have accordingly collected the pancreatic juice from anesthetized dogs by placing a canula in the duct, and the secretion so obtained was diluted with equal volumes of 0.85 per cent. salt solution and injected into the femoral or external jugular veins of the same or other dogs. Varying with the anesthetic used and directly with the blood pressure level at the time of injection, we have obtained falls of from 20 to 60 mm. Hg by injecting the equivalent of 1 c.c. of pancreatic juice. The action does not appear to depend on any primary cardiac effect. The fall is prompt and recovery to the original level occurs within 2 minutes. Pan-

creatic juice from which the coagulable proteins were removed by heat caused an equally marked fall in the blood pressure.

Extracts of the fresh pancreas were also prepared with the Buchner press or by simple grinding in a mortar with sand or glass and shaking with 0.85 per cent. salt solution. As a general rule 3 c.c. of the resulting solutions representing 1 gm. of the organ were used for single injections. The results were similar whether the solution was used at once or was kept on ice for 18 to 24 hours. Before use the solutions were cleared as far as possible by centrifugalization and filtering. Such extracts produced a fall in blood pressure averaging 30 to 50 mm. Hg. Similar results were obtained by using extracts from which the coagulable proteid was removed by heat and acetic acid, and also with the alcoholic precipitate of such a solution taken up in the original volume of 0.85 per cent. salt solution.

The alcoholic filtrates of the preceding extracts evaporated to dryness at 37.5° C. and made up to the original volume are inert. Washed or unwashed organs give extracts of similar depressor activity. The clear solutions obtained after heating and acidification were used to determine the effects of the injection of small amounts of extract over long periods of time. Death resulted generally from the injection of the equivalent of 3.5 to 6 grams of pancreas over a period of about one half hour, but in one instance an animal received the equivalent of 31 grams of pancreas, during two and one half hours, before death occurred.

It was found impossible by continuous injection of small quantities to keep the blood pressure at the low level produced by a single injection of the equivalent of 1 gram of pancreas. The blood pressure remains but slightly below its original level, but if a large dose is then given there is a marked fall, with recovery to the level existing at the time of injection. Attempts to determine the effects of continuous absorption of pancreatic juice from the peritoneal cavity by causing leakage from the severed ducts were also unsuccessful.

That there might be some ground for drawing analogies between experimental findings and clinical conditions, extracts were prepared from human pancreas and it was found that their action on the dog was in every way similar to that obtained with

the pancreas of the dog. We have, however, from our experiments no evidence to show that the prostration and collapse of acute pancreatic disease are caused by the absorption and effect on the blood pressure of the unaltered pancreatic juice or the products of the earlier stages of autolysis. The part played by autolysis in the production of further depressor and toxic substances and especially the part played by the activation of the pancreatic secretion by the enterokinase, which body presumably finds entrance into the organ in the development of the human lesion, are phases of the subject which we now are investigating.

57 (582)

The significance of the structure of the medullary loop of the renal tubule of mammalia.

By **G. CARL HUBER.**

[*Laboratory of Histology and Embryology, University of Michigan.*]

A method of maceration has been devised recently[1] by means of which it is possible to isolate the entire renal tubule of adult mammals. Certain of the renal tubules thus isolated have been stained and permanently mounted in glycerine. This enables a study of their form in a manner hitherto not possible and admits of an accurate determination of their epithelial lining. Each mammalian renal tubule possesses four types of epithelium: (1) The pavement epithelium surrounding the glomerulus and lining the glomerular capsule; (2) the specific renal epithelium of the proximal convoluted portion and its medullary segment; (3) the pavement epithelium of the medullary loop; (4) the cubic or short columnar epithelium of the ascending or distal arm of the medullary loop and the distal convoluted portion. The following table shows the distribution of the last three types of epithelium in renal tubules of the rabbit, the tubules selected representing A, a tubule with renal corpuscle situated at the periphery of the cortex; B, a tubule with renal corpuscle situated in the deeper portion of the outer half of the cortex; C, a tubule the renal corpuscle of which is situated in the deepest part of the cortex.

[1] G. Carl Huber, "A method for isolating the renal tubules of mammalia," *Anat. Record.*, v., April, 1911.

	A, mm.	B, mm.	C, mm.
Proximal convoluted portion with medullary segment..... (Specific renal epithelium of cortex.)	11.3	9.4	10.2
Thin segment of medullary loop..... (Pavement epithelium.)	1.4	6.7	15.0
Thick segment of medullary loop and distal convoluted portion..... (Cubic or short columnar epithelium.)	7.8	6.9	3.6
Total length of tubules, exclusive of collecting duct.	20.5	23.0	28.8

The tubules selected represent type tubules and were selected with a view of drawing attention to the fact that the character of the tubule, and perhaps also its functions, changes with the position of the renal corpuscle in the cortex. Tubules with renal corpuscles situated near the periphery of the cortex possess short medullary loops with short segments lined by pavement epithelium. The deeper in the cortex the renal corpuscle is situated the longer becomes the medullary loop and the longer the segment of the loop lined by pavement epithelium. These statements pertain not only to the renal tubules of rabbits but also to those of other mammals including man. It may be observed from a study of the table that that portion of the renal tubule lined by the specific renal epithelium of the cortex, namely the proximal convoluted portion and its medullary segment, does not vary materially in length in tubules of different types. If one may assume a specific excretion for the special renal epithelium of the proximal convoluted portions and their medullary segments, it is evident that this specific excretion would be essentially of the same extent for all the renal tubules, irrespective of type. If, on the other hand, one may assume an absorption of water and perhaps certain salts in the thin segments lined by the clear, pavement epithelium the extent of this absorption must differ widely in tubular segments having this structure, and that, therefore, urine of different degrees of concentration must as a consequence enter the collecting ducts.

58 (583)

On fundamental changes in the action of some alkaloids upon frogs after cardiectomy or ligation of one aorta.

By **S. J. MELTZER.**

[*From the Department of Physiology and Pharmacology of the Rockefeller Institute for Medical Research.*]

At the last meeting of the American Physiological Society I reported that in cardiectomized frogs an injection of adrenalin will still cause dilatation of the pupil and an injection of strychnine will cause a tetanus. At the February meeting of this society I reported on the action of morphin on cardiectomized frogs. An injection of 10 or 15 mgr. of morphin in living, normal, medium-sized frogs causes at first no perceptible effect; after a few days a tetanus may set in, while there is at no time a depression. In cardiectomized frogs, however, an injection of 6 or 8 mgr. of morphin causes the appearance of tetanic symptoms in less than an hour. When 10 to 20 mgr. are injected the tetanus is preceded by a paretic state. After an injection of 25 to 30 mgr. the animal becomes paralyzed very early, to be interrupted later by only short weak tetanic movements.

I have since found that also after the ligation of one aorta, especially of the right one, a tetanus will very frequently develop two or three hours after an injection of 10 or 15 mgr. of morphin; in some cases the tetanus may persist as long as twenty hours. In some cases two or three hours after the development of the spasmodic state the tetanus became temporarily interrupted by paralysis which lasted only 30 to 60 minutes.

Since the beginning of April it often became difficult to obtain in cardiectomized frogs a tetanus; even with small doses of morphin the effect was in many cases complete paralysis, which set in very early after the injection. The same was true also of frogs with one aorta ligated. Here the heart might be seen beating forcefully for hours while the animal is completely paralyzed. The fact reminds one of the behavior of nerve and muscle irritability in spring frogs.

Finally in a small percentage of cardiectomized frogs curare

(curarin) was found to produce tetanic symptoms. It was found further that when the right aorta has been ligated and the tissues of one leg or of both were firmly contracted, under the exclusion of the sciatic nerves (Claude Bernard), curarin produced in most cases a stage of definitely increased reflexes and even of short tetanic attacks.

59 (584)

On the convulsant action of acid fuchsin (Abel and Barbour) in cardiectomized frogs.

By **DON R. JOSEPH** and **S. J. MELTZER.**

[*From the Department of Physiology and Pharmacology of the Rockefeller Institute for Medical Research.*]

In experiments carried out in this laboratory it was shown that in the absence of the cardiovascular mechanism alkaloidal solutions are well distributed through the body; that, for instance, injections of adrenalin cause dilatation of the pupil and injections of strychnine cause the development of spasms. It was further found that the action of some substances may be even greatly accelerated and more effective in cardiectomized than in normal frogs. Morphine, for instance, may cause a tetanus in 40 to 50 minutes.

In a recent communication of Abel and Barbour it was reported that an injection of a comparatively large dose (one mgr. and more per grm. body-weight) of acid fuchsin into a frog may cause after many hours (even as much as 20) the appearance of a series of convulsions. These investigators discovered further that in frogs in which the anterior third of the cerebral lobes was removed, such convulsions may appear very soon, 13 minutes and less, after an injection of only a small dose of the fuchsin, *e. g.*, 0.35 mgr. per grm. body-weight. With the consent of Professor Abel we investigated the behavior of fuchsin in cardiectomized frogs in which the brain remained intact. The following is a brief preliminary report of the results.

Observations were made so far on about forty frogs. In all cases in which fuchsin was injected into the dorsal lymph sac of cardiectomized frogs, convulsions never failed to appear and the time of appearance was never longer than half an hour after the

injection. In 27 frogs the injected dose of fuchsin was less than 0.1 mgr. per grm. of body-weight. In eighteen frogs the dose was 0.05 mgm. per gram body-weight, the time of onset of convulsions varying between 4 and 15 minutes. In some of these cases the entire dose for the frog amounted to less than one milligram of the fuchsin. In a few frogs the effective dose was not more than 0.025 mgm. per gram body-weight.

We have here another instance in which the action of a substance is greatly accelerated and much more effective in animals without a circulation than in normal animals. The experiments seem to show further that the minimum toxic dose of fuchsin is for cardiectomized frogs much smaller than even for frogs with the anterior part of the brain removed.

60 (585)

On the presence of dextrose in the exudate of pulmonary edema.

By **I. S. KLEINER** and **S. J. MELTZER.**

[*From the Department of Physiology and Pharmacology of the Rockefeller Institute for Medical Research.*]

Injection of adrenalin causes glycosuria and hyperglycemia. In some instances the animals which receive adrenalin die of pulmonary edema. The chemical composition of the exudate of pulmonary edema has never been investigated. An examination of the exudate of rabbits which died from pulmonary edema after receiving adrenalin revealed the presence of a considerable amount of dextrose. The causation of pulmonary edema by the injection of adrenalin is, however, a matter of mere accident and cannot be relied upon in a systematic study. After various attempts we found that inhalation of ammonia can be fairly well relied upon to produce edema and produce it in a quantity sufficient to make a quantitative test for a reducing substance. The exudate did not clot, which shows that no pure blood was mixed with it. The number of experiments, although not yet large, permits a definite preliminary report. Besides analyzing the pulmonary exudate, in most cases a quantitative analysis of the blood for reducing substances was made and in some instances also of the urine. Pulmonary edema was also produced in two normal ani-

mals and in one animal which received an intravenous infusion of dextrose. The results may be briefly summarized as follows: The exudate of pulmonary edema contains dextrose or a reducing substance. The concentration seems to be in general equal to that of the blood. Two hours or longer after an intramuscular injection of adrenalin the exudate of the pulmonary edema may contain 0.5 per cent. and more of dextrose, a quantity, which, at least so far, usually slightly exceeded that of the corresponding blood.

61 (586)

The effect of culture medium contaminated with the excretion products of Paramæcium on its rate of reproduction.

By **LORANDE LOSS WOODRUFF.**

[*From the Sheffield Biological Laboratory of Yale University.*]

A summary is presented of the initial experiments of a series which is planned to elucidate, if possible, some of the complex factors at work in a "hay infusion"; for example, such as those which determine the inter-dependence of the organisms, their sequence, time of appearance, disappearance, etc. The data outlined were derived from the study of: (*a*) the effect of different volumes (2, 5, 20 and 40 drops) of culture medium on the rate of reproduction of *Paramaecium;* (*b*) the effect of changing the culture medium daily and on alternate days on the rate of reproduction of *Paramaecium;* and (*c*) the effect of culture medium, in which large numbers of paramæcia have been living, on the rate of reproduction of *Paramaecium.* It is believed that the results obtained justify the following conclusions:

1. The rate of reproduction of *Paramaceium aurelia* and *Paramaecium caudatum* is influenced by the volume of the culture medium, within the limits tested, and the greater the volume the more rapid is the rate of division.

2. Paramæcia excrete substances which are toxic to themselves when present in their environment, and these substances are more effective when the organisms are confined in limited volumes of culture fluid.

3. The excretion products of paramæcia play an appreciable part in determining the period of maximum numbers, rate of decline, etc., of this animal in "hay infusions."

62 (587)

On the origin of glycocoll in the animal body.

By **A. I. Ringer.**

[*From the Physiological Laboratory, Cornell University Medical College, New York City.*]

The various forms of protein, with the exception of casein and gelatin, contain about four per cent. of their N in the form of preformed glycocoll N. On giving benzoic acid or its salts to animals, they have the power of detoxicating it by combining it with glycocoll and forming hippuric acid.

About eleven years ago, Parker and Lusk employed this fact in an attempt to determine the maximum amount of hippuric acid that rabbits can produce. They studied the relationship between the total N in the urine and the glycocoll N eliminated as hippuric acid, HN/N. They found that for every 100 grams of total N excreted in the urine, an average of about 4 grams of N was eliminated in the glycocoll radical of the hippuric acid. After administering the first large dose of lithium benzoate to the animals, they invariably obtained a much higher HN/N ratio, 9.01 per cent. in experiment III., 7.14 per cent. in experiment IV., and 7.87 per cent. in experiment VI. They regarded this as a "sweeping out" of surplus glycocoll.

Since then, Wiechowski and Magnus-Levy have studied the same problem. Wiechowski obtained HN/N ratios of 45.4, 55, 50 and in one case, even as much as 64 per cent. Magnus-Levy found in his rabbits a maximum ratio of 25 and 28 per cent., in his sheep 27.8 per cent.

To investigate the cause of these discrepancies, Professor Lusk kindly suggested that I continue the study of this problem.

Experiment I.

A goat weighing 42.3 kg. was employed. In Table I., the results of this experiment are summarized. It shows that the animal was able to eliminate in its urine a good deal more glycocoll than is found preformed in the proteins of its tissues. The hippuric acid formation, *i. e.*, the glycocoll elimination does not depend upon the amount of protein catabolized, but within certain

TABLE I.

Date, 1910.	Period.	Weight.	Total N. Gr.	Hippuric Acid, Gr.	Hippuric Acid Nitrogen, Gr.	Hippuric Acid N % / Total N	A Benzoic Acid Fed.	B Benzoic Acid Excreted as Hippuric Acid.	A/B Per Cent.	Hippuric Acid per Kg. Weight.	Remarks.
February 25	I.	——	5.441	——	——	——	——	——	——	——	——
February 26	II.	——	6.39	——	——	——	——	——	——	——	——
February 27	III.	——	5.027	——	——	——	——	——	——	——	——
February 28	I.	42.3	4.303	10.19	0.805	18.7	8.47	6.95	82.04	0.247	250 gm. hay, 100 gm. oats.
March 1	II.	——	Urine Lost			23.06	16.94	——	——	——	88 gm. hay, 195 gm. white bread, 190 gm. milk.
March 2	III.	41.25	7.23	19.46	1.537	21.26	16.94	13.27	78.32	——	200 gm. white bread, 150 gm. hay.
March 3	IV.	——	9.08	27.75	2.193	24.14	21.18	18.93	89.39	——	100 gm. bread, 100 gm. cabbage, 160 gm. carrots.
March 4	V.	40.1	6.45	11.06	0.874	13.54	8.47	7.54	89.06	——	Ate what was left from previous day, no more.
March 5	VI.	——	6.36	30.93	2.443	38.41	25.42	21.10	83.05	0.786	Starving.
March 6	VII.	39.35	8.06	28.28	2.234	27.72	25.42	19.03	74.86	——	Starving.
March 7	VIII.	——	7.81	15.59	1.230	15.77	25.42	10.63	41.83	——	Starving. All benzoate one dose.
					Intermission of sixteen days.						
March 23	IX.	——	8.12	19.96	1.577	19.41	42.36	13.61	32.15	——	Starving.

limits runs parallel to the amount of benzoic acid fed. In period VI., it reached its maximum. 38.4 per cent. of the total N was eliminated as glycocoll N in the hippuric acid.

Where do these enormous quantities of glycocoll originate? It might be argued that the source of the glycocoll found in the system lies in the different vegetable foods which are known to contain rather large quantities of free amino-bodies. But after giving 8 grams of sodium benzoate to a calf two weeks old which had had no other form of food but milk, the protein of which contains no glycocoll, it was found that it was able to form and eliminate hippuric acid glycocoll as readily as the adult goat.

Ingestion of glycolic or glyoxylic acid with very large quantities of sodium benzoate in rabbits, does not increase the quantity of glycocoll formed as indicated by the amount of hippuric acid eliminated. This might have been possible from their chemical relationship to glycocoll. (A detailed account of these experiments will be given in the final report.)

On careful consideration of the nitrogen metabolism in animals during the benzoate period, a possible explanation of the origin of glycocoll suggests itself. The goat during the three days of the foreperiod, excreted an average of 5.6 grams of N. Excepting the first day, which may be due to a sudden change in the quantity of food, there is a marked rise in the protein metabolism throughout the course of the benzoate period. This was observed in all the experiments and in all the varieties of animals that have been experimented upon. Furthermore, the increase in the protein destruction, *i. e.*, that increase in the N elimination above the normal, or above a previous day of a smaller benzoate dose, is always two to three times greater than the amount of nitrogen that has been eliminated as glycocoll in the hippuric acid molecule.

In Table II., the results of experiments on two rabbits are recorded. These show that the amount of urea N plus ammonia N during the benzoate period does not differ from normal days, and also that the extra N catabolized is much greater than the amount of N that was eliminated in the form of hippuric acid.

All these facts suggest the possibility that the glycocoll excreted as hippuric acid does not come from the fraction of protein that would have been metabolized had no benzoate been given,

but entirely from the extra protein which is destroyed, due to the presence of the toxic substance. We cannot state with any degree of certainty, what the character of the intermediary processes is, but that it is specific and peculiar seems very probable, for none of the extra N destroyed goes over into urea. It is all eliminated either as glycocoll or as undetermined N.

TABLE II.

Rabbit No. 6.

Date, 1911.	Period.	Weight.	Total N.	Urea N.	Per Cent. of Total N.	NH_3N.	Per Cent. of Total N.	Hippuric Acid, N.	Per Cent. of Total N.	Benzoic Acid Fed.
March 12	I.	1.8	0.802	0.7024	87.6	0.232	2.89	—	—	—
March 13	II.	1.72	1.147	0.796	69.4	0.36	3.177	0.088	7.7	1.7
March 14	III.	1.60	0.913	0.782	85.67	0.166	1.82	—	—	—

Rabbit No. 7.

March 23	I.[1]	1.96	0.596	0.487	81.68	0.043	7.23	—	—	—
March 24	II.	1.71	1.369	0.9673	70.66	0.1127	8.23	0.082	6.0	1.7
March 25	III.	1.68	1.199	1.0898[2]						

63 (588)

Experiments on the diffusibility of alkaloids through rubber.[3]

By **J. B. SIDBURY** and **WILLIAM J. GIES.**

[*From the Laboratory of Biological Chemistry, of Columbia University, at the College of Physicians and Surgeons, New York.*]

Rosenbloom and Gies have found that various ether-soluble substances, when dissolved in ether and placed in rubber bags immersed in ether, readily pass through the rubber membranes

[1] Period of about 12 hours.

[2] Urea + NH_3N = 90.9 per cent.

[3] This study is one of a projected series on *physico-chemical conditions in the cell*, which in turn constitutes a section of a comprehensive plan of research on the composition of protoplasm as well as the structural and dynamic relationships of cell constituents and products. These investigations are now in progress in the Laboratory of Biological Chemistry of Columbia University, at the College of Physicians and Surgeons, and under the auspices of the George Crocker Special Research Fund.

thus imposed.[1] We have found that various alkaloids and some related substances readily diffuse through rubber under such conditions.

Our experiments were conducted as follows: A moderate quantity of the pure ether-soluble substance was mixed with 15–25 c.c. of ether.[2] This mixture was poured through a funnel into a new air-tight rubber condom in such a way as to preclude the possibility of overflow upon the external surface. The bag was then immersed in about 50 c.c. of ether in a narrow salt-mouth bottle 7 inches high. With the bag suspended at full extension in this position, its mouth was about an inch above the opening in the bottle. The protruding condom was supported in the neck of the bottle by a tightly fitting cork stopper, which also served to keep the bag closed. After a diffusion period of convenient length (sometimes 2 to 5 days),[3] the condom was removed from the bottle, the ether diffusate was poured into a porcelain dish, and the ether completely removed by evaporation on a steam bath. At least one appropriate test was then applied to the residue.[4]

Meanwhile, the ether solution in the condom was removed. A large volume of water was then poured into the suspended bag, which, during its distention by the water, was carefully examined for signs of leakage. In a few instances defective membranes temporarily rendered the outcome doubtful. All results with such bags were ignored, of course. Each of the tests, even after reliable positive responses, was repeated at least once with a *new* rubber bag.

The substances named below (the complete list of those already tested in this connection) are readily diffusible under the conditions of these experiments:

A. Apomorphin, atropin, brucin, caffein, cocain, codein, colchicin, coniin, morphin, narcein, narcotin, nicotin, physostigmin, quinin, strychnin, veratrin.

[1] Rosenbloom and Gies, *Journal of Biological Chemistry*, 1911, ix; *Proceedings of the American Society of Biological Chemists*, p. xiv (December, 1910); also, PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE, 1911, viii, p. 71.

[2] Substances which did not dissolve readily were triturated with ether in a mortar.

[3] Some of the alkaloids pass through rubber almost immediately under the conditions of these experiments.

[4] In the experiments with nicotin, the "tobacco odor" of the concentrated liquids was very pronounced.

B. Acetanilid, antipyrin, phenacetin, picric acid, picrotoxin, pyramidon, salicylic acid.

Experiments with other solvents, and with additional substances of alkaloidal type, will be added to this series.

64 (589)

Notes on Fischer's theory of the influence of acids in the production of edema.[1]

By **F. G. GOODRIDGE** and **WILLIAM J. GIES.**

[*From the Laboratory of Biological Chemistry, of Columbia University, at the College of Physicians and Surgeons, New York.*]

Several years ago Gies published some of the results of a preliminary study of the effects of acids on tendon collagen.[2] Last December Kantor and Gies reported their observation that collagen fibers from tendon immediately swell markedly in *free* acid but do not swell at all in any strength of *combined* acid[3]—facts on which they base a new microscopic test for free acid. These results naturally led Kantor and Gies to consider the relation of such facts to Fischer's theory of edema, which they were investigating at the time these observations were made. Lately we have gone into this particular phase of the matter with some experiments on fibrin. Similar experiments are under way with other colloids and with various tissues.

Fischer's general conclusion in regard to edema is stated in the following terms:[4]

"A state of œdema is induced whenever, in the presence of an adequate supply of water, the affinity of the colloids of the tissues for water in increased above that which we are pleased to call normal. The accumulation of acids within the tissues;

[1] This study is one of a projected series on *proteins and their combining qualities*. which in turn constitutes a section of a comprehensive plan of research on the composition of protoplasm as well as the structural and dynamic relationships of cell constituents and products. These investigations are now in progress in the Laboratory of Biological Chemistry of Columbia University, at the College of Physicians and Surgeons, and under the auspices of the George Crocker Special Research Fund.

[2] Gies, *Science*, 1907, xxv, p. 462.

[3] Kantor and Gies, *Journal of Biological Chemistry*, 1911, ix; *Proceedings of the American Society of Biological Chemists*, p. xvii.

[4] Fischer, "Edema — a study of the physiology and the pathology of water absorption by the living organism," 1909, p. 99.

brought about either through their abnormal production, or through the inadequate removal of such as some consider normally produced in the tissues, is chiefly responsible for this increase in the affinity of the colloids for water, *though the possibility of explaining at least some of the increased affinity for water through the production or accumulation of substances which affect the colloids in a way similar to acids or through the conversion of colloids having but little affinity for water into such as have a greater affinity must also be borne in mind.*" [1]

Fischer's views on the influence of acid in the production of pathological edemas appear to us to be over-emphasized. Special stress was laid by Fischer upon lactic acid as a causative factor in pathological edema, but apparently no experiments were performed with that acid. The action of electrolytes on the power of lactic acid to excite water absorption by colloids was not ascertained. Acids such as hydrochloric were Fischer's chief reliance in his experiments with acids.

We find, when moist shreds of fibrin are severally suspended in gelatin solution, peptone solution, fresh egg white, blood, milk, and meat juice, that hydrochloric acid solution (0.2 per cent. to 10 per cent.) may be added to the mixture in each case *in any proportion* without inducing visible effects on the fibrin shreds, *unless sufficient acid is added to provide an excess in the* FREE *state.* Very large quantities of acid may be added to such mixtures without appreciable bloating effect.[2] If the colloids in the artificial solutions and protoplasmic liquids enumerated above are combined with any proportion of the acid up to exactly their *maximum* affinity for it (hydrochloric acid), so that the liquids while strongly acid to litmus respond negatively to tests for *free* acid, then moist fibrin shreds can be kept in such acid fluids indefinitely without swelling to any perceptible degree. Warm concentrated gelatin solutions may be put into these conditions of free and combined acidity. After such solutions have been permitted to gelatinize, moist fibrin shreds which have been imbedded in the resultant jellies swell perceptibly, provided the gelatinized mass contains *free* acid, *but the shreds do not appear to absorb water from the medium if its contained acid is only in* COMBINED *form.* It is obvious that such

[1] Special emphasis is laid by us on the part of the quotation which we have italicized.

[2] Similar observations have been made with alkaline mixtures. We expect to describe, in the near future, a new test for free alkali based upon the behavior of fibrin in association with dissolved protein in alkaline media.

facts have an important bearing on any theory of acid causation of edema.

Our experiments do not permit us to deny that acids may be influential factors in the causation of edematous processes. Our results emphasize the fact, however, that the acids which may be produced in, or that are carried into, tissues tend to unite there with non-colloidal basic radicals and with dissolved colloids before combining with suspended colloids. The chemical means and excretory processes by which *living* protoplasm maintains a state of reaction-constancy cannot easily be overcome. In Fischer's published experiments on the bloating effects of acids, *large* excesses of *free* acid were present in all but a few cases. Would Fischer contend that edematous tissues contain *free* acid?

We feel that acids are not the only causes of colloidal water absorption in edema. Results obtained by Berg and Gies[1] several years ago indicate that *enzymes* facilitate any such influence that acids, whether free or combined, may exert; and vice versa. Fischer himself alludes, "in passing" (p. 109), to a result in harmony with that view. The italicized portion of the foregoing quotation from Fischer's book is broad enough to include enzyme influences and all other contributory factors. Experiments along these lines are still in progress.

65 (590)

The relation of the toxic dose of horse serum to the protective dose of atropin in anaphylaxis.

By **HOWARD T. KARSNER** and **JOHN B. NUTT.**

[*From the McManes Laboratory of Pathology, University of Pennsylvania.*]

This study was prompted by the publications of Auer and Lewis,[2] and of Auer,[3] which definitely demonstrated the prophylactic action of atropin sulphat in the asphyxia of immediate anaphylaxis. The results of these writers have been confirmed repeatedly.

[1] Berg and Gies, *Journal of Biological Chemistry*, 1907, ii, pp. 508 and 522.

[2] Auer and Lewis, *Jour. A. M. A.*, 1909, viii, 458; *Jour. Exp. Med.*, 1910, xii, 153; *ibid.*, p. 165.

[3] Auer, *Amer. Jour. of Physiology*, 1910, xxvi, 439.

In our own studies we used guinea pigs averaging about 400 grams in weight and sensitized by subcutaneous administration of 0.05 c.c. horse serum. The atropin was injected intravenously five minutes before the toxic dose of horse serum which also was administered into the jugular vein.

The study shows that as the toxic dose of horse serum is increased the protecting dose of atropin must also be increased, but the increase in protecting dose is not proportionate to that of the horse serum. The curve of protecting dose rises much more sharply than that of horse serum and finally a point is reached where the animal succumbs to the dose of atropin. A 400-gram guinea pig is killed almost instantly by a dose of 0.060 gram atropin.

That the effect of the atropin is physiological and not due to any alkaloidal combination with the toxic fraction of the horse serum is shown by the fact that a mixture of atropin and horse serum incubated at 37° C. and dialyzed for four days killed sensitized animals whereas a control in the same proportions but not dialyzed saved the animals from anaphylactic death.

66 (591)

The effect of specific vaccines in the typhoid of rats and mice.

By **EDNA STEINHARDT** and **THOMAS FLOURNOY.**

[*From the Laboratory of Hygiene, University of Michigan, and the Pathological Laboratory, Bellevue Hospital.*]

Owing to the impossibility of infecting small laboratory animals by feeding with typhoid bacilli, the immunity produced by specific vaccines has always been tested by subcutaneous or intra-peritoneal inoculations of the living culture.

These methods do not produce a disease comparable to human typhoid, but when rats and mice are fed with certain of the paratyphoid group they contract a disease whose pathology does closely resemble it; therefore these were used for the comparative study of the vaccines. The Danysz virus (one of the Gaertner group) was the test organism.

White mice were used in the first series and the vaccines tested were killed cultures, Vaughan's residue, sensitized bacilli (Bes-

redka's vaccine) and protective inoculations of serum, all the injections being given subcutaneously to make them more comparable with those in man. When later, these vaccinated mice were fed with living cultures, no protection was shown, sickness usually fatal occurred exactly as in the untreated cases. After an interval, all of the mice that recovered were re-fed with the living culture and all contracted the disease again, showing that no immunity had been established.

Rats being less susceptible than mice, the experiments were repeated upon them. The vaccinated animals when fed with living cultures were not protected but those which had been previously fed with small doses of the living virus, were completely immune to subsequent feeding with large doses. Experiments are now being carried out to see if these rats are also immune against intra-peritoneal and sub-cutaneous inoculations and if it is a strictly specific immunity.

Experiments on treatment, specific and otherwise, gave negative result in mice; in rats the investigations are still being carried on.

67 (592)

The relationship of the left suprarenal capsule to the sugar production of the liver in dogs.

By **J. J. R. MACLEOD, C. D. CHRISTIE** and **R. G. PEARCE.**

[*From the Physiological Laboratory, Western Reserve University, Cleveland, O.*]

We have obtained certain results bearing on the relationship between the left suprarenal capsule and the left splanchnic nerve-control of the glycogenic function of the liver which, in view of the topical interest in this subject, we believe it well to report at present, especially since it will take some considerable time to complete the investigation of which they form a part. The percentage amount of reducing substance in the blood of the inferior vena cava, opposite the hepatic veins, was determined by Reid's method at varying periods after stimulation of the left splanchnic nerve. In the first series of results given in the table the left suprarenal capsule was intact; in the second series this gland was excised.

TABLE.

PER CENT. REDUCING SUBSTANCE IN BLOOD OF VENA CAVA INFERIOR OPPOSITE ENTRANCE OF HEPATIC VEINS IN DOGS FED WITH EXCESS OF CARBOHYDRATE.

A. *Without Excision of Suprarenal.*

No. of Expt.	Before Stimulating.	During Stimulation.	After stimulation Removed.	Remarks.
16	0.136	0.176 (7 min.)[1]	0.184 (10 min.)[2]	
18	0.124	0.110 (7 min.)	0.144 (30 min.)	No rise in
19	0.130	0.176 (10 min.)	0.148 (30 min.)	blood pressure.
20	0.199	0.265 (5 min.)	0.415 (30 min.)	
21	0.151	0.195 (7 min.)	0.168 (23 min.)	
24	0.132 0.121 (30 min. later)	0.154 (7 min.)	——	
25	0.194 0.213 (30 min. later)	0.280 (5 min.)	——	
32	0.111 0.122 (30 min. later)	0.166 (9 min.)	——	

B. *After Removal of Left Suprarenal Capsule.*

27	0.118	0.124 (10 min.) 0.109 (25 min.)	——	
28	0.120 0.082 (30 min. later)	0.101 (5 min.) 0.103 (20 min.)	——	
29	0.171 0.173 (30 min. later)	0.177 (16 min.)	——	
30	0.163 0.127 (30 min. later)	0.113 (15 min.)	——	
31	0.249 0.248 (25 min. later)	0.270 (5 min.) 0.256 (20 min.)	——	

In every case, but one (no. 18) in which the suprarenal was intact there was a distinct increase in the reducing power of the blood, but when the suprarenal was exsected no increase occurred (except a slight transitory increase in no. 31). The blood pressure rose in all cases except no. 18.

68 (593)

Comparison between the blood flow in the arm and in the hand.

By **A. W. HEWLETT** and **J. G. VAN ZWALUWENBURG.**

[*From the Department of Internal Medicine, University of Michigan.*]

In 1909 the authors published a plethysmograph method for determining the rate of blood flow in the arm (Heart, I., 87) The results obtained by this method for normal individuals, partly

[1] The time periods in brackets indicate when the specimens of blood were removed in (1) after the beginning of stimulation; in (2) after the stimulation had been discontinued.

or completely stripped to the waist, under the ordinary conditions of room temperature, usually lay between 2 and 5 c.c. of blood flow per 100 c.c. of arm substance per minute. More recently, by a calorimeter method, G. N. Stewart has obtained rates in the hand approximately 10 c.c. of blood flow per 100 c.c. of hand substance per minute. We have attempted to compare the two methods because it seemed improbable to us that this discrepancy could depend entirely upon variations in external conditions, such as the room temperatures or the amounts of clothing worn.

The plethysmograph method was applied to the hand and the results compared with those obtained by the calorimeter method on the hand and with those obtained when the plethysmograph included the forearm and the lower arm. Our method does not give very satisfactory results for the hand. The curves are apt to rise abruptly when the pressure is applied and the rate of swelling remains constant but a short time before it lessens, owing apparently to the small venous capacity of the hand.

It is nearly always possible, however, to obtain curves where the rate of inflow is nearly constant for 2 c.c. or more. By selecting such parts of the tracings, an approximate rate of flow in the hand was obtained.

TABLE I.

	Average Room Temp.	Average Calorimeter Temp.	Rate by Calorimeter.	Plethysmograph Plate.			Remarks.
				Hand.	1st Arm.	2d Arm.	
Co.	24°	25.7°	3.1	4.8	2.9	3.1	Thick skin, hand cold.
Ca.	23°	29.8°	4.8	7.8	2.3	2.4	Hand cool.
So.	27.5°	29.0°	10.0	10.2	4.5	4.5	Hand warm.

In the experiments charted in Table I. the individuals were stripped to the waist. The rate of flow in the hand was first determined by Stewart's method. After this the hand was dried and successive determinations of the blood flow, by the plethysmograph, were made on the forearm, the hand, and again on the forearm. A glance at this table indicates that the blood flow in the hand was relatively faster than it was in the combined hand, forearm, and lower arm; and, furthermore, that the plethysmograph method, when applied to the hand, gave somewhat faster rates of flow than did the calorimeter method. In these experiments the plethysmograph contained air only.

In order to compare the two methods more closely a second series of experiments were performed in which the individual remained clothed and the hand, or arm, in the plethysmograph was covered with water of the same temperature as the water that had been in the calorimeter. The results of these experiments, shown in Table II., agree with the first set.

TABLE II.

	Average Room Temp.	Average Calorimeter.	Rate by Calorimeter.	Plethysmograph Rate.	
				Hand.	Arm.
V.Z.	24.5°	29.8°	7.4	9.6	
V.Z.	24°	28.0°	7.4	8.3	
V.Z.	25.5°	29.6°	4.7	8.6	3.1

These observations indicate that the blood flow in the hand, relative to its volume, is faster than is the flow in the combined forearm and hand. We found considerable differences in the results obtained by the two methods used on the hand. We do not know the exact causes of these differences but are inclined to believe that both hand methods are subject to greater error than is the plethysmograph method when applied to the arm.

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On the phagocytic inclusion of carmine particles by sarcoma cells growing in vitro with consequent staining of the cell granules.

By **F. M. HANES** and **R. A. LAMBERT.**

[*From the Department of Pathology of the College of Physicians and Surgeons, Columbia University, New York.*]

The cells of rat and mouse sarcomata when cultivated in vitro show active amœboid movements and wander for considerable distances into the surrounding plasma. Upon adding finely powdered carmine particles to the plasma medium the wandering tumor cells take up the particles in an active phagocytic manner. The carmine particles within the cells are easily distinguishable by their opacity and angularity. The carmine is partially dissolved within the cell and brings into evidence the granules of the

cytoplasm. These granules are round, of very constant size and are stained pink by the carmine. They occupy the entire cytoplasm. By means of Altmann's fixative and stain exactly similar granular pictures are obtained.

70 (595)

The formation of metastases after an intravascular injection of tumor emulsions.

By **I. LEVIN** and **M. J. SITTENFIELD.**

[*From the Department of Pathology of Columbia University, George Crocker Special Research Fund, New York.*]

It is generally accepted that metastasis in malignant tumors is formed by the proliferation of tumor cells which have been transported to distant parts of the organism through the blood or lymph channels. The cells of the primary tumor penetrate in some manner into the lumen of the vessels, are swept away by currents as emboli, and finding lodgment in some distant part of the organism, they proliferate and form secondary tumors.

This conception of the formation of metastasis was established through observation of autopsy material and no direct experimental proof of the matter was adduced up to the present. All experiments with intravascular injection of human tumor material into animals either gave negative results or were entirely untrustworthy.

In all the extensive literature of the last decade on the subject of the transplantable cancer of the white mouse and rat there appears no statement in regard to intravascular injection of tumor material with the aim of forming metastasis. The only exception is a short note by Graf, who obtained negative results.

Metastasis in malignant tumors of the white mouse and rat occurs rarely as compared with human cancer, and the channels for the transportation of the tumor cells are in a majority of cases the blood vessels. The reason for the rare occurrence of metastasis in the rat and mouse Ehrlich, in accordance with his athreptic theory of immunity, sees in the fact that tumors in these animals are usually of extreme malignancy and grow to very large

size. The cells of the primary tumor use up all the specific food found in the organism of the host, and the cells transported from the primary tumor to other regions of the organism do not find the necessary nourishment and consequently can not proliferate. Carl Lewin on the other hand thinks that the fact that metastasis in these animals takes place only through the aid of the blood vessels and not the lymphatics, accounts for the rare occurrence of metastasis in these animals. As was shown by M. B. Schmidt through his observations on human cancer, blood is capable in a majority of cases of destroying cancer cells, found within the blood vessels.

The study of the influence of the blood upon cancer cells carried by it and the capacity of such cells to form metastasis may serve to clear up a number of phenomena in the genesis of cancer. In view of this a systematic study was undertaken by the writers on the experimental formation of metastasis, of which the present communication forms the first report.

The experiments consisted in an injection of a tumor emulsion into the jugular vein or carotid artery of a white rat, and were conducted on three different tumors.

Sarcoma of the White Rat.—This tumor is very malignant, grows to a large size, and the occurrence of metastasis after a subcutaneous inoculation is very rare. In hundreds of animals inoculated by the writers local dissemination was observed not more than in a half dozen cases, and once only was metastasis found in the liver. An emulsion of this tumor was prepared by cutting it in Haaland's mincing machine, grinding in normal salt solution and filtering through a layer of coarse gauze. The milky opalescent fluid contains a sufficient number of living cells to produce a tumor growth after a subcutaneous inoculation. Thirty-six rats received an injection of this emulsion into the jugular vein. The animals were killed at periods ranging from eight days to four weeks after the injection and a thorough search made in all the organs for metastasis. Not in a single instance was a metastatic tumor found. No microscopical study was made of the organs which appeared normal on gross inspection, since this investigation does not concern itself with the question, where the tumor cells circulating in the blood find lodgment, but whether such a

cell transported into a certain organ will form there a visible metastasis. All suspicious nodules found anywhere and all lungs appearing abnormal on gross inspection were examined microscopically with negative results. The same negative results were obtained in six rats, where the injection was made into the carotid artery, and the animals survived. This method is very difficult of execution and the animals usually die a few minutes after the injection from respiratory paralysis, while the heart continues its action a few minutes longer.

Carcinoma of the White Rat (Flexner-Jobling).—This tumor is not as malignant as the previous one, but metastasis occurs frequently after a subcutaneous inoculation. The metastasis is usually found in the lungs, but it was also observed by Flexner and Jobling in the kidney, the heart and even in the lymphatic glands, though the authors believe that in most cases the metastases were produced through the blood current. Sixteen rats received an injection of an emulsion of this tumor into the jugular vein. In three animals metastasis was found in the lungs, no other organ showed any metastasis. Six rats survived an injection into the carotid and of these animals one showed metastasis in the lungs. In another rat, in which the injection was made in the carotid against the stream of blood, the animal was found dead twelve days later. At the autopsy a nodule was found on the wall of the left ventricle. The animal remained dead in the cage over night and the specimen had greatly deteriorated, still the nodule resembled microscopically the picture described by Flexner and Jobling of a metastasis found by them in the heart.

Sarcoma of the White Mouse.—Ehrlich has shown, that when this tumor is inoculated subcutaneously into a rat, there forms a small nodule, which remains for 8–10 days, and is then absorbed. No metastasis formation of this mouse tumor into a rat was ever noted. An emulsion of this tumor was injected into the jugular vein of twelve rats. The rats were killed in periods of 4–8 days and in two of the animals metastatic nodules were found in the liver. Neither in the lungs nor in any other organ was there found any metastasis.

The results of these experiments seem to indicate in accordance with the opinion of M. B. Schmidt, that cancer cells introduced

into the blood circulation in a majority of cases lose their proliferating power. Ehrlich's opinion that the most virulent tumors do not form metastasis also seems to be correct, as no metastasis was formed after the injection of the most virulent sarcoma of the rat. But the athreptic theory does not explain this fact. None of these animals had any sarcoma growth anywhere and consequently the sarcoma cells introduced into the circulation could find all the necessary specific food.

The most interesting phenomenon observed in the course of these experiments consists in the fact, that while the Flexner tumor found lodgment in the lungs after an intravascular injection, the mouse sarcoma produced metastasis only in the liver. It would seem that the different topographic distribution of metastasis in the different kinds of malignant tumors is due not so much to the difference in the channels through which the cancer cells are transported, and which were identical in all experiments reported here, but to a specific affinity between cancer cells and cells of certain organs. The comprehension of this specific affinity between a cancer cell and a particular part of the organism of the host may be helpful in elucidating many factors in the genesis of tumors and will be the main object of the further study of the experimental metastasis formation by the writers.

SCIENTIFIC PROCEEDINGS.

ABSTRACTS OF THE COMMUNICATIONS.

Forty fourth meeting.

University and Bellevue Hospital Medical College. May 17, 1911.

71 (596)

The balance of acid-forming and base-forming elements in foods, and its relation to ammonia metabolism.

By **H. C. SHERMAN** and **A. O. GETTLER.**

[*From the Laboratory of Food Chemistry, Columbia University.*]

In continuation of previous work[1] ash analyses have been made of a number of foods and from the percentages of total sulphur, phosphorus and chlorine on the one hand, and of sodium, potasium, calcium and magnesium on the other, the excess of acid over base or of base over acid which will result from the oxidation of the food has been calculated. Previous ash analyses have also been studied and supplemented by such determinations as were necessary to permit the calculation of this balance for a wide range of food materials. Meats and eggs show a predominance of acid-forming elements; in fruits and vegetables the base-forming elements predominate. From this standpoint the fruits and vegetables tend to balance the meats of the diet. Milk and the cereals contain acid-forming and base-forming elements in more nearly equivalent proportions.

Through the kindness of Mr. L. H. Smith, samples of corn which had been bred through ten generations for high and low protein content respectively were obtained from the Illinois Agricultural Experiment Station. The ash-analyses of these were very similar except for the higher sulphur content of the high protein corn, which resulted in this sample showing a slight predominance of acid-forming elements, while in the low protein corn the base-forming elements predominated.

In order to determine to what extent the excess of acid brought

[1] Sherman and Sinclair, *Jour. Biol. Chem.*, Vol. III., 307.

into metabolism by the oxidation of the food is neutralized by ammonia in man, an experiment was made in which the influence upon ammonia excretion of a known change in the diet was studied quantitatively. The change of food consisted in substituting rice for potatoes in a simple mixed diet and (neglecting the feces but allowing for the unoxidized sulphur excreted during each period) was calculated as equivalent to the introduction of 28.3 c.c. normal acid per day. The increased ammonia excretion was equivalent to 10.7 c.c. normal acid per day. Thus, only about one third of the extra acid introduced by the change of food was eliminated as ammonia salt.

The authors take pleasure in acknowledging their indebtedness to Professor Mandel for the privilege of carrying on a part of the work in his laboratory at the University and Bellevue Hospital Medical College.

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The determination of small amounts of iodine in organic combination — A modification of Hunter's method.

By **E. C. KENDALL.**

[*From the Pathological Department of St. Luke's Hospital, New York, F. C. Wood, Director.*]

Hunter's method for the determination of iodine in organic combination consists of fusion of the organic matter, and the formation of sodium or potassium iodide. The iodide is oxidized to iodate with sodium hypochlorite, phosphoric acid is added, and the excess of chlorine is removed from solution by boiling. Potassium iodide is now added and each molecule of iodate liberates six atoms of iodine. The liberated iodine is titrated with sodium thiosulphate, the final reading being six times the amount of iodine originally present.

The removal of chlorine by boiling is a time-consuming and uncertain operation. The writer has modified the above method by removing the excess of chlorine from solution with phenol. The free chlorine adds directly to the benzol ring forming an unionized compound which does not interfere with subsequent operations. This modification makes the above method accurate and rapid.

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Beef extract as a "constant" culture medium for Paramæcium aurelia.

By **LORANDE LOSS WOODRUFF** and **GEORGE ALFRED BAITSELL.**

[*From the Sheffield Biological Laboratory of Yale University.*]

Previous work with pedigree cultures of *Paramœcium aurelia* and *caudatum* has apparently shown that the life history of these forms, when bred continuously on infusions of hay made up exactly the same from day to day, tends to run in a cycle which terminates with the death of the culture. Previous work (Woodruff) has also shown that *Paramœcium aurelia* may be bred indefinitely on a culture medium which is frequently varied.

In view of these results the following question is of interest: Is the longevity of paramæcia on a "varied environment" dependent upon the intrinsic stimuli from the frequent changes in the medium, or is a "constant" medium of hay infusion unfavorable because it lacks some elements which are essential for the continued existence of this protozoon? To test this point beef extract was chosen because it was believed that it contains all of the essential elements. After a series of preliminary tests, a .025 per cent. solution of Liebig's extract of beef was decided upon for the experiments.

Briefly, it may be stated that the solution of beef extract employed has proved (during the seven and one half months of the experiment) to be practically as favorable a medium for the reproduction of the pedigree culture of *Paramœcium aurelia* as the "varied" culture medium; and therefore the conclusion seems justified that this culture can, in all probability, be continued indefinitely on this medium, and that it is the "composition" of the medium rather than "changes" in the medium which is conducive to the unlimited development of this culture without conjugation or artificial stimulation. It is also believed that a "constant" medium of beef extract, in this or similar solutions, will prove to be a favorable culture medium in which to breed pedigree cultures of paramæcia for certain physiological studies,

since it is clear from many investigations that the reactions of paramæcia to various stimuli are greatly modified by the past and present environment of the organisms.

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Conjugation of closely related individuals of Stylonychia.

By GEORGE ALFRED BAITSELL.

[*Sheffield Biological Laboratory of Yale University.*]

A "wild" *Stylonychia pustulata* was isolated from a laboratory aquarium on October 1, 1910, and placed on a "constant" beef extract medium. This culture, which consisted of four lines, was kept on depression slides and the animals were isolated daily. The medium proved to be a favorable one for this animal and in a period of about four months the culture reached the 350th generation. At that time (February 5, 1911), when for ten days they had been dividing at an average rate of over three divisions per day (which was the highest rate of division that they had attained) a considerable number of conjugations between closely related cells occurred in the "stock" of the culture left over from the daily isolations. For a period of about three weeks this phenomenon was quite general in the stock and apparently would occur whenever a sufficient number of animals were present on a slide. To study the effects of conjugation, 132 conjugating pairs were isolated. These were kept in exactly the same kind of medium as that in which the conjugation had occurred so that the character of their environment was not changed by the isolation. From over 90 per cent. of the isolated conjugating pairs, ex-conjugants were obtained (after a union of the usual duration) which were perfectly normal in general appearance and behavior. However, none of these ex-conjugants divided and none lived 48 hours after separating. Animals obtained from "split" conjugating pairs also died without dividing. It was impossible to prolong the life of the ex-conjugants by any of the methods tried. Also from the time that the epidemic of conjugation made its appearance there was a continuous and rapid fall in the division rate of the main lines of the culture when averaged for ten-day

periods, and by the 25th day following, the entire culture had died out. In another culture, which was started from this one at the 150th generation and kept on a hay infusion medium, conjugation did not occur and this culture is still alive.

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The cultivation of tissue in plasma from alien species.

By **ROBERT A. LAMBERT** and **FREDERIC M. HANES.**

[*From the Department of Pathology of the College of Physicians and Surgeons, Columbia University.*]

The present series of experiments have been concerned with the attempt at cultivating in vitro rat sarcoma, rat spleen and mouse carcinoma, in plasma obtained from animals of other species, and at analyzing the factors contributing to the phenomena observed.

In a former note we recorded the fact that mouse and rat plasma could be interchanged as culture media for the tumors of these species, but that growth seemed to be more vigorous when homologous plasma was used. Guinea pig, rabbit, dog, goat, human and pigeon plasmas have been employed in the studies herewith reported.

For determining the viability of tissue under the conditions of the experiment we have made transfers of the pieces to homologous plasma—a rapid and satisfactory test. Animal inoculations have also been made in the case of tumor tissue, but aside from the delay in noting the results the procedure has other objections.

As a culture medium for rat sarcoma guinea pig plasma is only slightly less suitable than rat plasma, the difference consisting chiefly in the extent of the out-wandering of cells. The cells may remain viable in a single drop of plasma for twelve days or more; we have had pieces which showed marked activity after a month's sojourn in several drops of the alien medium. Mouse carcinoma seems to grow almost as well in guinea pig plasma as in rat plasma. Mitoses have been observed after eight days, and cultures nine days old produced tumors when inoculated into mice.

Rabbit plasma is distinctly less suitable for the growth of mouse

and rat tumors. Liquefaction about the pieces of tissue is often quite marked. With sarcoma the growth, though relatively slow, may continue for ten to twelve days.

In dog plasma pieces of sarcoma, after one or two days, present a fairly diffuse radial outgrowth of clear spindle cells, which after this time undergo rapid disintegration. About the pieces there is noted a narrow clear zone (liquefaction). With mouse carcinoma liquefaction is more marked and there is little or no outwandering of cells.

We have not observed any of the phenomena of growth when using goat plasma. After a few hours there is seen surrounding the pieces of tissue a wide granular zone. Liquefaction of fibrin does not occur.

In human plasma the most striking and constant phenomenon is the progressive liquefaction of the fibrin, which is practically complete after six or seven days. In spite of the loss of framework there takes place, however, an active migration of cells, which wander out along the cover glass, reaching often the edge of the medium. As a rule the cells move out separately, but we have observed in several preparations of rat spleen radial out-growths simulating the appearance seen in homologous plasma where the fibrin network is preserved. In the single cells attached to the cover glass the most interesting changes have been noted, especially in the cultivation of rat spleen. After four or five days cells of extraordinary size begin to appear, reaching a maximum size in two or three days. Such a "giant cell" examined in the fresh state shows about its centre a clear nuclear area, with or without knob-like prominences, surrounded by a highly granular zone, which in turn merges into a clear filmy indefinite protoplasm with processes. In their entire extent these cells vary from 100 to 700 mikra in diameter. When stained the knob-like prominences are seen to be nuclei with distinct nuclear membranes and prominent nucleoli. These nuclei are often seen in close apposition arranged about a light hematoxylin staining, reticular or vacuolated area. In the fresh this portion of the cell was interpreted as nucleus. Many large cells varying considerably from these were also seen. Quite large multinucleated cells have been observed in the growth of sarcoma and carcinoma in rat plasma but none that simulated

those just described. Pieces of rat spleen or tumor when transferred to rat plasma after five or six days in human plasma begin to grow very actively. The cells, however, often present extremely ragged outlines. Giant cell formation may be noted after the transfer.

The growth of rat sarcoma in pigeon plasma is especially beautiful and appears to be quite characteristic. During the first four or five days there is a progressive radial extension of large clear spindle cells of strikingly uniform size and morphology. The strings of cells are connected by long slender processes. Fine granular fat accumulations in the protoplasm appear early and increase in size and number with the age of the specimen, just as occurs in rat and guinea pig plasmas. As a rule no further growth takes place after the fifth day, and the cells at this time begin to show signs of disintegration. Intact well stained nuclei, however, may sometimes be seen in preparations eight or nine days old. Transfer of the pieces of tissue in four-day specimens to fresh pigeon plasma does not result in a prolongation of the period of activity.

In summarizing the above findings we notice that goat plasma is the only medium used in which there was no growth; in dog plasma growth was of short duration. Arranged in the order of suitability we have guinea pig, rabbit, pigeon, human, dog and goat. In a study of cytolysins for rat and mouse tissues we found that hemolysins were present in human, dog and goat sera. The sera tested were taken from animals from which plasma had been obtained for tissue cultivation. We have found also that in plasma from guinea pigs immunized against rat corpuscles, growth of rat sarcoma does not occur, or is of an abortive character, and that there is a similar inhibition of growth in plasma from guinea pigs previously treated with large doses of rat sarcoma.

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The behavior of fat-soluble dyes in the organism.

By **LAFAYETTE B. MENDEL** and **AMY L. DANIELS.**

[*From the Laboratory of Physiological Chemistry, Sheffield Scientific School, Yale University, New Haven, Conn.*]

It is well known that the fat-soluble dye, Sudan III., is readily deposited in the adipose tissue of animals. An attempt was made by the authors to study the movements of the dye under conditions where fat transport takes place (*e. g.*, in starvation, phlorhizin- and phosphorus poisoning). The dye readily migrates into the blood with the fat under these conditions, but is rarely found in the liver tissue into which large quantities of fat enter (fatty infiltration). This is explained by the observation that the Sudan III. is abundantly excreted with the bile into the intestine from which it may be reabsorbed. Sudan III., which is insoluble in water, is not excreted through the kidneys except where alimentary lipuria is induced (in rabbits and rats). The elimination from the liver is not accomplished through the solvent medium of fat excreted in the bile (lipocholia); but the dye is soluble in bile as well as in solution of the isolated bile salts. *We have thus established a path of elimination for fat-soluble (or bile-soluble) substances through the biliary secretion.* An investigation of a considerable number of water-insoluble, fat-soluble compounds—mostly non-toxic aniline dyes and food colors—showed comparable conditions justifying the above general conclusion. It has further been established that these water-insoluble compounds do not experience absorption from the intestine in the absence of bile. Dissolved in fat-emulsion and introduced into the organism by alimentary, subcutaneous, or intravenous paths, these dyes are always eliminated with the bile into the intestine. When there is a paucity of fat in the diet the fat-soluble dyes may be absorbed through the agency of reabsorbed bile, but they are speedily eliminated again by the liver channels; with an abundance of fat to act as carrier, they travel with it through the lymphatics into the circulation. The distribution of fat-soluble dyes within the organism depends on the presence of fat and its migrations.

Thus they may be carried to or from adipose tissues, be deposited in the egg-yolk, or be secreted in company with fat in the milk of animals; they apparently do not traverse the placenta. The dyes have not been detected in the lipoids of the nervous tissue. We have failed to note any inability on the part of animals to utilize fats in which Sudan III. has been deposited.

77 (602)

Experimental studies on creatine and creatinine.

By **W. C. ROSE.**

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The excretion of creatine induced by starvation in rabbits, is inhibited partially or completely by feeding a diet of carbohydrates alone. The creatine elimination is not reduced by feeding a diet of fat alone or by a diet of fat and protein.

Experimental interference with carbohydrate metabolism leads to the elimination of creatine. After phlorhizin diabetes, which depletes the store of carbohydrates, and during phosphorus poisoning, which disturbs the glycogenic functions, the output of creatine in dogs is decidedly increased.

An increase in the output of creatine plus creatinine (total creatinine) is always accompanied by an increase in total nitrogen elimination. This parallelism in inanition and with nitrogen-free diets, is ascribed to a common source,—namely, true tissue or endogenous metabolism. The metabolism of exogenous or reserve proteins is not accompanied by the production of creatine or creatinine.

Coincident with the increased elimination of total creatinine during fasting, a significant increase in the creatine content of muscle occurs in rabbits and hens. This indicates an increased production of creatine during the accelerated catabolic processes.

Creatine is a normal constituent of the urine of the young until the age of puberty. Possibly this is due to insufficient glycogenic functions. Though no direct evidence for such an assumption has been obtained, still the ease with which children develop

glycosuria and acidosis, and the rapidity with which they succumb to diabetes, renders such an explanation probable. It is conceivable also that during the period of growth the demand for carbohydrates for the histogenetic processes may be so great that the cells are left in partial carbohydrate hunger, and are unable to perform the "endo-catabolic" activities as perfectly as in later life.

Without question the metabolism of creatine is intimately associated with carbohydrate metabolism.

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The rate of tumor growth in underfed hosts.

By **PEYTON ROUS.**

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Workers with transmissible neoplasms have had frequent occasion to observe that sick or emaciated animals are relatively resistant as hosts for implanted tumor. It does not develop in them with the same readiness as in healthy individuals. A kindred phenomenon has been noted by Moreschi[1] in studying the relation of nutrition to tumor growth. He found that in mice losing weight on a low diet an engrafted sarcoma survived with less frequency and grew more slowly than in the well-fed controls. Indeed these controls died of their tumor sooner than did the fasting animals.

This being so might it not be possible to delay by food-restriction the course of inoperable tumors? And might not the development of metastasis after excision of a primary growth be hindered by the same means? In an attempt to answer these questions the author has performed a series of experiments with the Flexner-Jobling adeno-carcinoma of the rat. This neoplasm in its invasive spread and tendency to metastasize has a striking likeness to some of the cancers of human beings.

A bread compounded of oatmeal, rye-flour, corn-meal, milk and sugar, was baked in large quantity, dried, ground, and, with sufficient milk to moisten it, was used as the sole food of the

[1] C. Moreschi, *Zeitschr. f. Immunitätsforsch.*, 1909, VI., 651.

experiment animals. Preliminary observations were made on several series of healthy rats to determine how little of the bread would sustain life while permitting of a gradual emaciation, and then 110 young, growing animals, carrying the Flexner-Jobling tumor, were submitted, half to food restriction, while the other half were fed full and used as controls. The food was carefully measured out, the exact size of the tumors charted each week, and the rats weighed twice a week. Moreschi observed the effect of food restriction from the time of the tumor's implantation or shortly thereafter until the host's death. For our purpose animals were mainly used in which the tumor had attained a large size before the restricted diet was begun. Contrary to expectation it was found that these large tumors continued to grow with the same rapidity in hosts emaciating on a restricted diet as in the controls, many of which were still gaining weight. With yet larger tumors and hosts already cachectic food-restriction had also no effect on the primary growth, and none on the frequency of metastasis formation.

Moreschi's findings were confirmed so far as regards the influence of poor nourishment of the host to hinder the development of tumor grafts. And when food restriction was begun a few days (four) after the introduction of the grafts it was noted that the tumors developed a little more slowly than in the controls. It is easy to understand how, given such a retarding influence on the tumors early in their career, the experiment animals might outlive the controls, as Moreschi found to be the case. The sarcoma he used was probably more susceptible than our carcinoma to changes in the host's nutrition.

The fact that large tumors take their course irrespective of the condition of nourishment of the host does not at present admit of direct explanation any more than does the phenomenon of regeneration in starving animals. It is to be correlated with the clinical observation on malignant disease that the tumor often grows with great rapidity despite a marked progressive emaciation of the patient. Our experiments have not yet answered the important question as to whether in animals from which the primary tumor has been removed the metastases will behave like tumor grafts in that their growth is slower in ill-nourished hosts; or

whether, as is the case with large primary tumors at this time, the growth will go on independently of the host's condition of nourisment.

79 (604)

Preliminary report upon the transmission of haemolysins from mother to offspring.

By **L. W. FAMULENER.**

[*From the Research Laboratory, Department of Health, New York City.*]

In view of the fact that little work has been done upon the transmission of hæmolysins from mother to offspring, and that there is a lack of agreement between the reported results of the workers in this field, the subject was taken up for further investigation. The question is of practical importance, since a parallel relationship exists between hæmolysins and bacteriolytic bodies: the latter group of substances play a more or less important rôle in immunity against certain infections.

Goats were selected as the most suitable experimental animals for these studies. In each case the animal was actively immunized by repeated injections of washed sheep-corpuscles given subcutaneously. Serum and milk samples were collected, throughout each experiment, and stored in an ice-box. All were tested at the same time, under uniform conditions, after the given experiment was closed.

One series of animals were immunized immediately following the birth of their young. In all, excepting one case, the milk contained no demonstrable hæmolysins. As evident, the sucklings from the mothers which supplied the negative milk, gave negative sera. The suckling which received the mother's milk containing hæmolysins, showed no specific hæmolysins at any time in its serum. The hæmolysins did not appear (by test) in the milk in this case until about one week after birth of the young.

A second series of animals were immunized at different periods during the course of gestation. Before birth of the young the animal's nipples were sealed to prevent the young getting any

milk before blood samples were taken. When twins came, one was placed, at once, upon cow's milk for control. At the time of birth none of the young showed specific hæmolysins in the blood serum. But those getting the colostrum and first milk rapidly acquired, and retained, the specific antibodies. The colostrum in those cases was very rich in hæmolysins, but the antibodies disappeared from the milk output after a few days, in so far as we were able to ascertain.

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The effects of intraperitoneal injections of adrenalin on the partition of nitrogen in the urine of dogs.

By **JACOB ROSENBLOOM** and **WILLIAM WEINBERGER.**

[*From the Laboratory of Biological Chemistry of Columbia University, at the College of Physicians and Surgeons, New York.*]

Underhill and Closson have shown that the *subcutaneous* injection of adrenalin chlorid solutions into dogs is not attended by any significant change in the proportion of the urea, ammonia and creatinin nitrogen of the urine.

In our work we used two specimens of the colorless adrenalin chloride, 1:1000, of Parke, Davis and Co. They were purchased in the open market. Each was tested for its pressor action at the conclusion of the corresponding injection experiments and was then found to be practically as active as ever.

The metabolism experiments were carried out by the methods in use in this laboratory.

Intraperitoneal injection of adrenalin chloride solutions was without effect on the proportions of nitrogen in the forms of urea, ammonia, creatin and creatinin, purins and allantoin.

In one adrenalin injection period of eighteen days, a total of 62 c.c. of a 1:10,000 adrenalin solution was given intraperitoneally and in another injection period of six days a total of 29 c.c. of a 1:1000 adrenalin chloride solution was administered.

The following table shows the different percentages of nitrogen for the several experimental periods:

PERCENTAGE OF TOTAL NITROGEN.

Periods.	Urea N, per cent.	Ammonia N, per cent.	Creatin and Creatinin N, per cent.	Purin N, per cent.	Undetermined N, per cent.
I. (Fore period).....	88.5	3.64	2.51	0.16	5.19
II. (First injection period)	85.7	3.75	2.50	0.20	7.86
III. (First post injection period).....	86.5	3.61	3.33	0.24	6.32
IV. (Fore period).....	86.7	3.65	3.20	0.19	6.26
V. (Second injection period)	87.0	3.92	3.73	0.16	5.19
VI. (Second post injection period).....	84.2	4.29	3.62	0.17	7.72

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Experiments on the diffusibility of cholesterol-esters and of lecithan compounds.[1]

By **ERNST BOAS** and **JACOB ROSENBLOOM.**

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I. CHOLESTEROL ESTERS.

It has been shown in this laboratory that ether solutions of various biological substances pass through rubber membranes into ether.

We have found that cholesterol-benzoate, cholesterol-stearate, cholesterol-oleate and cholesterol-palmitate dissolved in ether will readily diffuse through rubber into ether.

Cholesterol-stearate with a molecular weight of 652.61 diffuses, whereas the various lecithans, with molecular weights considered to be 770 to 785, do not. If we assume that the diffusion of a substance depends on the size of its molecules, the above facts strengthen Hiestand's conclusion that the molecular weight of egg-yolk lecithin is 1446, which figure he obtained by a molecular weight determination.

[1] This study is one of a projected series on *lipins*, which in turn constitutes a section of a comprehensive plan of research on the composition of protoplasm as well as the structural and dynamic relationships of cell constituents and products. These investigations are now in progress in the Laboratory of Biological Chemistry of Columbia University, at the College of Physicians and Surgeons, and under the auspices of the George Crocker Special Research Fund.

II. LECITHAN COMPOUNDS.

Koch[1] has lately described the preparation of various compounds with lecithans, but it is uncertain whether these compounds are colloidal mixtures, mechanical mixtures or true chemical compounds. It seemed of interest to study the behavior of these substances in ether solution, when subjected to dialysis in rubber bags suspended in ether.

The preparations used in these experiments were made according to the method described by Koch. For the dialysis tests the solutions of the lecithan compounds were evaporated to dryness at 38° and the residues ground up with ether. The extracts were filtered and the filtrates placed inside of rubber bags and dialyzed against ether for thirty-seven days. The dialysates were tested every week to see if the substance combined with the lecithan diffused.

Compounds of lecithin with glucose, lactic acid, strychnin, digitonin, salicin, urea, creatin, creatinin and caffein were prepared. It was found that the glucose and lactic acid dialyzed completely, the strychnin, digitonin and salicin dialyzed partially, while urea, creatin, creatinin and caffein did not dialyze at all.

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The relative importance of stroma and parenchyma in the growth of certain organs in culture media.

By **MOYER S. FLEISHER** and **LEO LOEB**.

[*From the Departments of Pathology of the Barnard Free Skin and Cancer Hospital and the City Hospital of St. Louis, Mo.*][2]

In experiments carried out about fifteen years ago, one of us observed that during the regeneration of skin, the epithelial cells are able to penetrate into and to grow in coagula of blood and of blood plasma.[3] This suggested to him that it might be

[1] Koch, *Journ. Pharm. and Exp. Ther.*, 1911, ii, p. 239.

[2] We wish to express our thanks to Dr. D. L. Harris, director of the Pathological Laboratory at the City Hospital, who put the facilities of his laboratory at our disposal at a time when our laboratory had not been finished; and also to Dr. M. G. Seelig, who very kindly assisted us in a number of our experiments.

[3] *Archiv f. Entwicklungsmechanik*, Bd. VI., 1898; *Johns Hopkins Hospital Bulletin*, January, 1898; *American Journal of Anatomy*, Vol. III., 1904.

possible to make various tissues grow in culture media outside of the body, in the thermostat, as well as inside the body, in the latter case the body acting as a thermostat. Inasmuch as he noted that the epithelial and also connective tissue cells grew preferably in contact with solid structures as fibres of fibrin, and into solid gelatinous material rather than into fluids he attributed stereotropic sensitiveness to various tissue cells, and he consequently employed more or less solid culture media as agar and coagulated blood serum for his various experiments. At first he carried out experiments in vitro as well as experiments in which the animal body acted as an incubator. Lack of the necessary facilities made it very soon necessary for him to limit himself to the latter kinds of experiments.[1]

To our knowledge in these our earlier experiments for the first time the attempt was recorded in the literature to grow tissues of higher animals under artificial conditions in environments that differ from those found in the body under natural conditions, to separate experimentally growing epithelial from connective tissue cells, and furthermore to study the influence of the addition of certain chemicals upon the growth of tissues.[2] Thus among other facts it was found that epithelial cells can grow in these gelatinous culture media even after addition of certain salts; that this growth can take place independently of connective tissue cells, that the epithelial cells may divide mitotically in the culture media, that they invade the coagulum through ameboid movements, that they have the power of phagocytosis, taking up into their body small particles of the culture medium;[3] that the growth ceases after this period of activity, and we explained on the basis of our observations certain phenomena of cancer growth as due to active ameboid ingrowth of cancer cells into the deeper tissues.[4] Later Harrison[5] showed in most interesting experiments that it is possible to grow embryonic nervous tissue of the frog in a mixture of fibrin and serum of the frog lymph, and recently Burrows

[1] Chicago, 1897; *Archiv f. Entwicklungsmechanik*, Bd. XIII., 1902. *The Journal of Medical Research*, Vol. VIII., 1902; *Journal Am. Med. Association*, 1901.

[2] *Zeitschrift f. Krebsforschung*, Bd. V., 1907, pp. 14 and 15.

[3] *Archiv f. Entwicklungsmechanik*, Bd. XIII., 1902.

[4] *Johns Hopkins Hospital Bulletin*, January, 1898.

[5] PROCEEDINGS SOC. EXP. BIOL. AND MED., Vol. III., 1907; *Journal Experimental Zool.*, Vol. IX., 1910.

extended in Harrison's laboratory the use of fibrin and blood serum as a culture medium to the growth of various mammalian tissues. Other investigators especially Carrel and his collaborators have within the last nine months taken up these studies and grown tissues in fibrin as well as in agar. We also resumed recently our former experiments and here we wish to report briefly a few of our results as far as they concern the relative importance of stroma and parenchyma in the tissue growth in gelatinous culture media:

A. After transplantation in vitro as well as in vivo, the central parts of stroma and parenchyma become necrotic, only the peripheral parts remaining alive. The extent of this central necrosis varies in different organs; it is, for instance, greater in the case of the kidney than in the case of the testicle.

B. In regard to the relative importance of stroma and parenchyma in the growth of certain organs in coagula we notice certain differences in various organs.

1. In the ovary of the guinea pig and rabbit either the whole piece becomes necrotic or in other cases the connective tissue and some follicles of the cortex remain entirely or partly alive. The epithelial covering of the ovary remained alive and even proliferated in a few cases. Mitoses in stroma or parenchyma cells or distinct ingrowth of connective tissue or epithelium into the coagulum were not observed. The greater part of the tissue became necrotic.

2. In the case of the testicle of the rabbit the peripheral alveoli usually remain alive without however any new formation of spermatozoa taking place. An irregular development of some epithelial cells into multinucleated giant cells may occasionally be observed. An outgrowth of the parenchyma into the coagulum does not occur, while the connective tissue can grow very actively into the coagulum.

3. In the case of the kidney an outgrowth of connective tissue into the coagulum was a very frequent occurrence especially in cases in which in the periphery of the transplanted piece a part of the connective tissue capsule had remained adherent to the parenchyma. The tubular epithelium in the periphery of the transplanted piece usually remains alive. Growth in the parenchyma takes principally place within the area of the transplanted

tissue, the regenerating cells of the tubules pushing the older cells into the lumen of the tubule and such desquamated necrotic cells glue together and form casts. Tubular epithelium can also grow between the transplanted piece of kindey and the coagulum and sometimes it penetrates into the coagulum forming occasionally canals in the latter. Mitoses are seen in the proliferating cells of parenchyma and stroma and such cells dividing mitotically may be seen lying directly in the coagulum. This description holds good for the ordinary manner of experimentation. Under certain conditions which we expect soon to describe in more detail it seems possible to increase very markedly the proliferating and infiltrating activity of the tubules.

4. In the case of the mammary carcinoma of the mouse the growth of the parencyhma is very much more prominent than in the case of the normal organs examined, a fact that is in accordance with the rapid growth of carcinoma cells in contact with animal cells in the body.

5. While various authors state that after the ordinary transplantation of carcinoma of the mouse into the subcutaneous tissue the stroma perishes and only the parenchyma remains alive, after transplantation into the culture media the stroma of mouse carcinoma remains alive and shows even certain growth phenomena. This observation should suggest a renewed investigation of the fate of the stroma after transplantation of carcinoma into the subcutaneous tissue of an animal.

6. Both parenchyma and stroma of the carcinoma of the mouse grow approximately equally well in coagulated blood plasma of the rat and of the rabbit.

7. The parenchyma cells of carcinoma penetrate very much more frequently into the coagulum than the parenchyma cells of other organs investigated so far. Mitoses are frequently seen in the carcinomatous cells and they may even be found in cells lying in the coagulum.

8. In regard to the skin we observed in our early experiments published elsewhwere the epithelial cells to penetrate in relatively large masses into the coagulum and we furthermore described at that time mitoses in these infiltrating epithelial cells.

C. We also noticed that the epithelial cells of the skin may

become phagocytic and take up small particles of agar and coagulated blood serum. Similar observations we made recently in the case of growing tubular cells of the kidney and of carcinomatous cells[1] and probably also in the case of ingrowing stroma cells.

D. While the parenchymatous cells penetrate onto the coagulum in a more or less definite arrangement which to some extent corresponds to the normal arrangement of the parenchyma cell, the form of cell columns or of tubules being retained respectively, the connective tissue cells on the other hand grow as single cells often sending out long drawn out stellate processes in the coagulum, the various connective tissue cells being loosely connected by such processes. This arrangement enables the stroma cells to penetrate into the coagulum with relatively greater ease than is possible in the case of parenchyma cells.

E. Stroma as well as parenchyma cells (including carcinoma cells) show a definite direction in which they grow into the coagulum, both having the tendency to proceed along the fibers which form in the coagulum and to follow, if at all, for a short distance only a course vertical to the direction of the fibers in the coagulum. The connective tissue cells seem however in consequence of their isolated and independent mode of growth and of moving occasionally to be able to penetrate into the coagulum in other directions more easily than parenchyma cells, but they also usually follow the road of least resistance.

F. We recognize therefore variations in the relative importance of stroma and parenchyma in the case of the growth of different organs. In a provisional way we may assume that the parenchyma of those organs or tissues that normally show an additive (expansive) growth (active outgrowth) like carcinoma and stratified epithelium of the skin show likewise an infiltrative growth in coagula (in gelatinous culture media generally) and that those organs that normally or during regeneration do not show additive (active expansive out) growth, but rather compensatory hypertrophy, do correspondingly not show a strong tendency to infiltrative growth, when growing in culture media (testicle, ovaries). In the latter class however the stroma may or may not show infiltrative

[1] The same observation has been recently made in the case of tumor cells by R. A. Lambert and F. M. Hanes, Journal Exp. Med., Vol. XIII, 1911, p. 495.

growth. Certain other organs like kidney apparently hold an intermediate position. Here both parenchyma and stroma may grow into the culture medium; the growth of the parenchyma is however under ordinary conditions relatively slight.

There are however in all probability other factors of importance besides the one just mentioned. Thus we observed so far in our experiments a very much more active growth of the connective tissue of the testicle than of the ovary. Perhaps the difference in texture of the organs is in this case one of the determining factors, the looser texture of the testicle being more favorable to the outgrowth of the stroma than the dense structure of the ovary.

We furthermore found differences and similarities in the manner of growth of stroma and parenchyma in the coagulum.

83 (608)

A possible significance of the Cammidge reaction.

By **L. B. STOOKEY.**

[*From the Physiological Laboratory, Medical School, University of Southern California.*]

Smolenski[1] attributes the Cammidge reaction to saccharose. This led us to think of some intestinal lesion as a possible source of the Cammidge reaction. Two possibilities seem to be evident (1) absorption of saccharose as such, (2) reversible action of intestinal saccharase.

To test this view the Cammidge test was made on urines in cases of "chronic intestinal disturbance." Twelve cases, only one of which showed a clinical suspicion of a pancreatitis, were studied. Five gave a positive Cammidge reaction. The case showing probably the most pronounced reaction failed to give the Cammidge test after 48 hours' starvation. During the twelve hours following the starvation period a liberal quantity of milk sweetened with levulose was given. This did not lead to a positive Cammidge.

From the experiments made thus far it seems probable that in cases showing a positive Cammidge there may be some relationship between the amount of cane sugar ingested and the intensity of the Cammidge reaction.

[1] *Zeitschrift für physiol. chem.*, 51, p. 127.

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The relation of the fixation reaction to specific precipitation.

By **FREDERICK P. GAY.**

[*University of California.*]

This work represents a continuation of studies on the mechanism of the fixation reaction between a serum and its antiserum begun by the author in 1905. It was shown that this fixation which had been attributed by Gengou to the presence of anti-albuminous sensitizers was apparently produced by the precipitate formed in the serum-anti-serum mixture. A prolific literature has since sprung up engaged principally in establishing the presence or absence of parallelism between fixation and precipitation. The results in either direction are far from conclusive.

In the present work an attempt has been made to settle the question by studying more attentively an instance in which both precipitation and fixation are known to occur, and where one may reasonably be associated with the other. It is found that in addition to the voluminous precipitate which is known to be formed by mixture of an excess of the antiserum with the antigenic serum, there is another precipitate produced with certain individual antisera in the presence of a large excess of antigen. This latter, and we believe newly recognized, zone of precipitation lies above the zone of inhibition produced, as is well known, by the ordinary excess of antigen. The upper zone precipitate differs in its granular appearance and slowness of formation from the lower zone precipitate.

No fixation of alexin occurs as a rule in mixtures representing the inhibition zone and never in presence of the upper-zone precipitate which may, however, equal or exceed in volume a precipitate of the lower zone which produces complete fixation. This fixation in the lower zone precipitate mixture is produced almost invariably by the washed precipitate and not by the supernatant fluid. The latter fluid may, however, in some cases give partial fixation.

It is well recognized that the addition of a small amount of antigen to the formed precipitate will dissolve it. This applies

to the lower zone precipitate only. A solution of the precipitate by this method gives a perfectly clear solution which fixes as well as the formed precipitate. In similar manner solution of the precipitate by traces of acid or of alkali gives a fixing fluid equal in potency to the undissolved precipitate; the latter method is, for technical reasons, less conclusive than the former method of solution.

The conclusion to be drawn from this work is that alexin fixation by a mixture of serum and antiserum is produced by an antigen-antibody complex distinct from precipitinogen-precipitin but usually brought down by the precipitate in its formation in such a way as to give the appearance that the fixation is produced by the precipitate itself. It would seem then that Gengou's original supposition, without direct proof, of anti-albuminous sensitizers more correctly explains this type of the fixation reaction. The best expression of the conditions as they seem to exist would be found, however, in Nicolle's hypothesis who concludes that antisera contain two classes of antibodies, "coagulins" and "lysins," the former, in this particular instance, producing precipitation, and the latter, the fixation reaction.

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598. [with **G. A. Baitsell.**] Beef extract as a "constant" culture medium for *Paramæcium aurelia.*

EXECUTIVE PROCEEDINGS.

Fortieth meeting.

College of Physicians and Surgeons, Columbia University, October 19, 1910. President Morgan in the chair.

Members present: Atkinson, Auer, Banzhaf, Beebe, Benedict, Berg, Calkins, Field, Goldfarb, Hatcher, Hunter, Jackson, Joseph, Lee, Levene, Levin, Loeb, J., Mandel, J. A., McClendon, Meltzer, Meyer, Morgan, Murlin, Norris, Opitz, Ringer, van Slyke, Stewart, H. A., Stockard, Storey, Swift, Terry, Wadsworth, Wallace, Weil.

Members elected: John Howland, R. A. Lambert, Ernst Sachs, H. B. Williams.

Forty first meeting.

The Rockefeller Institute for Medical Research, December 21, 1910. President Morgan in the chair.

Members present: Auer, Dochez Emerson, Janeway, Joseph, Gies, Hawk, Lamar, Lusk, Meltzer, Sachs, Steinhardt, Stockard, Swift, van Slyke, Wallace, Wolf.

Members elected: F. J. Birchard, Charles B. Fitzpatrick, Charles E. A. Winslow.

Forty second meeting.

Cornell University Medical College, February 15, 1910. President Morgan in the chair.

Members present: I. Adler, H. M. Adler, Auer, Banzhaf, Beebe, S. R. Benedict, Calkins, Dochez, Dunham, Ewing, Famulener, Field, Gies, Joseph, Lambert, Lusk, J. A. Mandel, A. R. Mandel, Meltzer, Morgan, Ringer, Sachs, Terry, van Slyke, Wallace, Weil, H. B. Williams, A. W. Williams.

Members elected: W. F. Longcope, F. M. McCrudden, H. O. Mosenthal, Jacob Rosenbloom.

Officers elected: President, Thomas H. Morgan; Vice-President, P. A. Levene; Secretary, George B. Wallace; Treasurer, Graham Lusk.

Forty third meeting.

University of Pennsylvania, Laboratory for Hygiene, April 19, 1911. President Morgan in the chair.

Members present: Abbott, Auer, Cook, Donaldson, Eisenbrey, Goldfarb, Hatai, Huber, Joseph Levin, Lillie, Longcope, Lusk, Mandel, Meltzer, Morgan, Pearce, Reichert, Ringer, Taylor, Terry, Wallace, Wolf.

Members elected: Alfred E. Cohen, T. H. Montgomery.

Forty fourth meeting.

University and Bellevue Hospital Medical College. May 17, 1911. President Morgan in the Chair.

Members present: Auer, Benedict, Cole, Famulener, Field, Foster, Gies, Jackson, Joseph, Lambert, Levin, MacCallum, Mandel, Meltzer, Mendel, Morgan, Pappenheimer, Ringer, Rosenbloom, Rous, Sherman, Storey, Steinhardt, Swift, Terry, Wallace.

Members elected: F. W. Bancroft, D. H. Bergey, W. H. Frost, J. S. Githens, H. T. Karsner, I. S. Kleiner, W. H. Manwaring, E. B. Meigs, R. Ottenberg.

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PROCEEDINGS

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SCIENTIFIC PROCEEDINGS.

1 (610)

Experimental pernicious anemia.

By HERMAN M. ADLER.

[*From the Laboratory of the Danvers State Hospital.*]

These experiments were performed on rabbits; fourteen rabbits in all were employed. Olive oil was fed in varying doses. Two feedings of 10 c.c. of olive oil per kilo of body weight sufficed to kill after 5 days. 6 c.c. per kilo weight killed in six days when fed daily. 5 c.c. per kilo weight did not kill but produced secondary anemia with blood crises presenting the picture of pernicious anemia—blood count 4 to 5,000,000, Hb. 50 per cent. or less, achromia, irregularity in size and shape, polychromatophilia, stipling, blasts.

Eight rabbits were thus subjected to chronic poisoning with olive oil. Of these, three had previously been daily fed (for about a year) 0.3 gram quinine. Three rabbits had been treated for three months with intravenous injection of 0.01 gram quinine daily. The remaining two received daily feedings of olive oil without previous treatment. In all of these rabbits the blood picture of secondary anemia developed within a few days. In four the blood picture of pernicious anemia developed in from 2–3 months. The anemia was not constant but varied considerably. The weight curve approximately followed the appearance of the blood; dropping sharply as the anemia became apparent, rising as the anemia improved. Five of these rabbits showed marked impairment of nutrition during the entire course, the younger ones being decidedly stunted in their development. The three rabbits that had been fed quinine per os showed less severe disturbances and none of them have thus far (after 1½ years) shown the picture of pernicious anemia. The weight and blood

conditions improved markedly when the feeding of oil was suspended.

Calcium lactate, lime water, Fowler's solution and tincture of ferric chloride were fed at different times with the olive oil without, however, appreciably diminishing the effect of the oil. The action of the olive oil depends upon its content of triolein (oleic acid). Pure oleic acid was fed to a rabbit and found to be no more toxic than olive oil.

A noteworthy phenomenon in the chronically poisoned rabbits was a swelling of the heels, evidently hematomata, which came on suddenly in all of the rabbits, furthermore a crusted suppurating skin lesion which appeared on the inner surfaces of the ears of six out of eight of the rabbits.

The post mortem examination of the rabbits which died after only a few doses showed practically nothing except a slight congestion of the small intestine. In the autopsies on rabbits which had received many doses general absence of fatty deposits together with fatty changes in liver, heart and kidneys and an atrophic condition of the spleen and lymphoid apparatus were noted.

From this and from the appearances observed in human beings suffering from pernicious anemia, it is fair to assume that the lymphoid tissue, particularly of the intestine, is chiefly concerned in handling the fat absorbed from the food by the intestine.

2 (611)

The influence of age on the symptoms following thyro-parathyroidectomy.

By **SUTHERLAND SIMPSON.**

[*From the Physiological Laboratory, Medical College, Cornell University, Ithaca, N. Y.*]

With regard to the question as to whether the thryoid and parathyroid glands become less and less essential to the organism as age advances there is some difference of opinion. Vincent and Jolly[1] found that in the various species of animals which they used (cats, dogs, foxes, monkeys, rats, guinea-pigs, rabbits), the symptoms following thyroidectomy and parathyroidectomy were

[1] Vincent and Jolly, *Jour. of Physiology*, 1904, xxxii, p. 80.

not influenced, to any extent, by age or sex. In the case of dogs the writer can corroborate this statement, but it appears to be otherwise in the sheep, as the following account of experiments on this animal will show.

In the course of another investigation[2] the thyroids were completely removed from eighteen lambs, from seven to eight months old, and twelve adult sheep, without, in the course of the six months which intervened between the operation and their slaughter, any apparent ill effects. There was no falling out of the wool, nor any of the other symptoms of myxœdema supposed to be associated with complete thyroidectomy, and several of the adults gave birth to full-time, and to all appearance, perfectly normal lambs.

From three of these lambs, at the age of two months, the thyroids were removed, the two external parathyroids being left behind, and from two others at the same age (also born of thyroidectomized mothers) all the thyroid and parathyroid tissue was taken away. The latter, in the course of ten and nineteen days respectively, developed typical and acute parathyroid tetany. In one, the first fit was fatal in less than an hour from the onset, the rectal temperature being 112° F. one minute after death, and in the other, which was killed during the fit, the thermometer reached 108.7° F. immediately before death.

The three from which the thyroids alone were removed developed into typical cretins.

In these three, about one year after the first operation, the two remaining external parathyroids were removed. As a result of this there followed what appeared to be some gastro-intestinal disturbance, and on three or four occasions, several weeks apart, each lasting about a week, some stiffness of the limbs, but nothing of the nature of acute tetany with rise of temperature, increased respiration, etc., which was so marked in the other two. These three sheep are still alive more than four months after the second operation.

In addition to the three above mentioned, other two adult sheep, aged two and seven years respectively, have had the com-

[2] Simpson and Hunter, *Quart. Jour. Exper. Physiol.*, 1911, iv, p. 340.

plete operation of thyro-parathyroidectomy performed on them and no noticeable symptoms have followed.

In the sheep therefore it would appear that both the thyroid and parathyroid glands are much more important organs in the young than in the adult animal, and consequently that they become functionally less active as age advances.

In relation to the influence of the parathyroids on calcium metabolism it is interesting to note that the two young lambs which showed acute parathyroid tetany were fed almost entirely on milk, rich in calcium salts, while the three which had the external parathyroids removed when they were about fourteen months old, and the other two parathyroidectomized adults, lived on a purely herbivorous diet in which potassium salts predominate. It may be, however, that in the young animal, where bone is being rapidly formed, the ratio between the demand for calcium and the supply is even greater than in the adult, although in the latter a far smaller quantity is being ingested.

In the case of the adult sheep the results of thyro-parathyroidectomy are in agreement with those of MacCallum[1] who from a similar operation found that "Practically no effect whatever was produced in these five sheep, although in at least three of them ample time elapsed for the development of symptoms." The other two died early of pneumonia, due probably to the administration of ether.

3 (612)

Peculiarity in the mode of entrance of the optic nerve into the eyeball in some rodents.

By **J. A. BADERTSCHER.**

[*From the Physiological Laboratory, Medical College, Cornell University, Ithaca, N. Y.*]

In the majority of animals the optic nerve enters the eyeball as a round compact bundle of nerve fibers and the optic disc is circular in outline or nearly so. While removing the eyes from the woodchuck and prairie dog for histological material, I observed that here there was an exception to the general rule in that the

[1] MacCallum, *Johns Hopkins Hosp. Bull.*, 1907, xviii, p. 335.

optic nerve became transformed into a band flattened antero-posteriorly before it entered the eyeball, and that the disc was linear instead of circular in outline. In these animals the disc consists of a long slender band, bending slightly dorsally in the center and gently widening at the ends. The absence of rods and cones along this band indicates that it is a true blind spot. As far as the investigation has been carried on, this form of optic disc was found to reach its greatest development in the prairie dog and has only been observed in members of the family Sciuridæ. Further investigation is necessary to show whether this peculiarity is limited to species of this family.

Johnson[1] has pointed out the presence of an elongated disc in the squirrel and marmot, but he makes no statement regarding the mode of entrance of the optic nerve into the bulbus oculi. As stated above, the optic nerve spreads out in an antero-posterior direction shortly after it enters the orbit and penetrates the coats of the eye in the region marked by the optic disc. In the woodchuck and prairie dog the flattened portion of the optic nerve is slightly concave on both the dorsal and ventral sides, and the latter is marked by a slight groove extending along the long axis of the nerve midway between the ends of the disc. The optic nerve from a short distance within the orbit to the chiasma has the usual cylindrical outline. This tendency of the nerve to bifurcate is also seen in the squirrel and chipmunk but it has not developed to the same extent as in the animals above mentioned.

In the rabbit's eye the optic nerve enters the eyeball as a cylindrical bundle of nerve fibers, from which one might expect the optic disc to be circular in outline, but this is not the case as has been observed by Johnson and figured by Haab.[2] The optic papilla is nearly circular but instead of the fibers radiating from it to the different parts of the retina they divide into two nearly equal portions which run in opposite directions toward the equator of the eyeball. From these two bands the fibers spread out to the different parts of the retina. The expansion of the optic nerve on the inside of the bulbus oculi of the rabbit, in contrast to its spreading out on the outside of the bulbus in some forms of the

[1] Johnson, *Phil. Trans. Roy. Soc.*, 1901, 194 B, p. 30.

[2] Haab, cited by Fuchs, "Physiologisches Praktikum für Mediziner," p. 227.

family Sciuridæ may suggest an intermediate stage in the process of evolution between the latter group and those higher mammals in which the fibers radiate to all parts of the retina from a circular optic disc. However, an extended investigation is necessary before any conclusion can be arrived at with regard to the possible taxonomic value of this character.

Experiments to trace the fibers of the optic tract are now in progress. As the optic nerve is spread out near the eyeball, it is a simple operation to cut either the inner or outer half for a study of the degeneration of its fibers.

So far I am not in a position to offer any opinion regarding the physiological significance of this peculiarity.

Note.—In the case of the squirrel and European marmot this peculiarity in the optic nerve is mentioned in Cuvier's "Leçons d'Anatomie Comparée," Tome 3B, p. 430.

4 (613)

Biological and toxicological studies upon *Penicillium puberulum* Bainier.

By **C. L. ALSBERG** and **O. F. BLACK.**

[*From the Bureau of Plant Industry, Washington, D. C.*]

This species when grown upon Raulin's solution, in pure culture, produces a new organic acid which has been termed penicillic acid. This acid gives a brownish-red solution when treated with a dilute solution of ferric-chloride. With ammonia it gives a deep red color. From the analyses, molecular weight determinations and other data, it seems probable that this acid belongs to the same general class of compounds as are found in lichens, and termed lichen acids. Like them, it is slightly bitter and irritating. Pharmacologically, it is moderately toxic, having an antiseptic action and being a protoplasmic poison. It is not astonishing to find substances of this class in fungi, since lichens are symbiotic forms, composed of fungi and algæ. The finding of this type of substances in the pure culture of a fungus makes it probable that in lichens, lichen acids are the product of the fungus metabolism, and not of that of the algæ.

5 (614)

The influence of the salts of calcium and potassium on the degree of hypertrophy produced by adrenalin injections in rabbits.

By **HUGH A. STEWART.**

[*From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York.*]

A series of experiments was conducted with a view to determine whether the antagonism between calcium and adrenalin, as recently described by Meltzer and Auer, Schrank and others, holds good also as regards the hypertrophy of the heart which adrenalin produces when injected intravenously into rabbits.

The method of investigation was as follows: One lot of rabbits was given every second day 0.2 c.c. of adrenalin chloride (1 in 1,000) in 2 c.c. of physiological salt solution. Throughout the whole series, twenty-four injections were given. A second lot received the same amount of adrenalin in 2 c.c. of a 10 per cent. solution of calcium chloride for the same length of time. Still a third lot was given 0.2 c.c. of adrenalin (1 in 1,000) in 2 c.c. of physiological salt solution every second day for twenty-four injections. The animals of this lot, however, were kept on a diet of carrots and potatoes only, the object being to give a diet containing a minimal amount of calcium. A fourth lot received the same amount of adrenalin in 2 c.c. of a 2 per cent. solution of potassium chloride.

As a result of these experiments, data were obtained which justified the following conclusions:

I. That calcium does not inhibit or prevent the hypertrophy of the heart produced by adrenalin.

II. That on a diet poor in calcium, the degree of hypertrophy is insignificant. From this it would seem that a certain percentage of calcium in the tissues is necessary in order that injections of adrenalin will produce hypertrophy.

III. The addition of potassium to adrenalin increases slightly the degree of hypertrophy.

6 (615)

Color inheritance in *Fundulus* hybrids.

By **FRANK W. BANCROFT.**

[*From the Rockfeller Institute for Medical Research.*]

In the hybrids of *Fundulus heteroclitus* and *Fundulus majalis* it was found that, in general, wherever the color characters were not concerned with the rate of development, the *F. heteroclitus* characters were dominant over those of *F. majalis*. These dominant characters were:

1. Red yolk chromatophores large and abundant.

2. Black yolk chromatophores large and usually polygonal in shape.

3. An early first crop of head chromatophores is present, in addition to a later crop which is found in both pure species and both hybrids.

4. There is a row of red chromatophores on the lateral line shortly before and immediately after hatching. As soon as the fish hatch the red chromatophores begin to fade, and within a few days disappear entirely.

On the other hand, when the characters were concerned with the rate of development, the Mendelian dominance was partly or entirely obscured. These characters were:

5. In *F. heteroclitus* there are no or very few black chromatophores on the lateral line at hatching; but they increase rapidly during the first few days after hatching. In *F. majalis* there are 50 or 60 black lateral line chromatophores at hatching. The hybrids are intermediate.

6. In *F. heteroclitus* when the yolk chromatophores first appear they are uniformly distributed over the whole yolk; while in *F. majalis* they are confined to the hemisphere containing the embryo. The hybrids are intermediate.

7. Both with respect to time, and with respect to the stage of development of the embryo the yolk chromatophores appear first in *F. heteroclitus*, next in the *F. heteroclitus* egg hybrid, next in the *F. majalis* egg hybrid, and last in the pure *F. majalis*.

An apparent case of blended inheritance in the time of the

first appearance of head pigment was found to be really a case of the combination of two crops of head chromatophores, one of which did not develop in the recessive species *F. majalis*.

7 (616)

A note on the relation of the semi-circular canals of the ear to the motor system.

By **J. GORDON WILSON** and **F. H. PIKE.**

[*From the Laboratories of the University of Chicago and Northwestern University.*]

The work on the semi-circular canals was undertaken with the object of studying: (1) The results of stimulation of the end organ of the vestibular nerve; (2) the immediate and remote results of destruction of the labyrinth or of the eighth nerve on one or both sides; (3) the effect of removal of various parts of the brain on phenomena which have been observed to follow stimulation or destruction of the labyrinth, and (4) the paths of nervous connection between the labyrinth and the eye muscles — the optico-acoustic path. Later it is the intention to study anatomically the degenerative processes occurring in various parts of the central nervous system after destruction of the labyrinth or eighth nerve.

Method.—The mastoid bone is exposed by a skin incision and the separation of the muscles from their attachment at the linea nuchæ superioris and the anterior inferior border of the pars mastoidea. A trephine opening is made through the outer table and diploe of the mastoid bone. The hard portion of the otic bone, in which the semi-circular canals are imbedded, can be removed with a mastoid gouge and the labyrinth destroyed without direct anatomical injury to the cochlea. Dogs, cats and tortoises were used. The results here given apply to dogs, unless otherwise stated.

Results.—Immediately following recovery from the anesthetic after removal of one labyrinth, there is marked torsion, particularly of the anterior part of the body, of the animal toward the injured side. The animal is unable to walk and falls over toward

the injured side on attempting to stand. It may roll completely over on the floor. There is a quick movement of the eyes toward the sound side and a slow return to the injured side. The unsteadiness of gait and the nystagmus are transient. Marked torsion of the head is permanent (two years).

Stimulation of the labyrinth, under moderate anesthesia, by putting hot water in the trephine opening before destruction of the semi-circular canals, causes slow marked deviation of both eyes to the opposite (unstimulated) side. Ice in the trephine hole causes the eyes to deviate to the same (stimulated) side. On electrical stimulation, the zinc terminal of a battery causes deviation to the opposite side; the carbon terminal, to the same side.

The deviation of the eyes on stimulation of the labyrinth may be obtained after total removal of the cerebrum, the optic thalamus, the anterior portion of the anterior corpora quadrigemina and the cerebellum. The torsion of the head in tortoises following destruction of one labyrinth is as marked after decerebration as before. There are no apparent "shock" phenomena in these reflexes after decerebration.

The nystagmus is not due to irritation of the wound, but to the action of the opposite uninjured labyrinth. Nystagmus following extirpation of the second labyrinth is much less marked, and more fleeting than after extirpation of the first, and is reversed in direction, *i. e.*, the quick movement is to the injured side.

There is no torsion of the body nor any rolling movements after extirpation of both labyrinths at the same operation, nor when the second labyrinth is destroyed after an interval. The animal becomes very ataxic and is unable to grasp food if both labyrinths are removed at the same time.

Complete or partial removal of the cerebellum one or two weeks previous to removal of one labyrinth has no effect upon the onset and course of labyrinthine nystagmus. Eye movements following injury to the cerebellum are jerky, irregular, and quick in all directions. Labyrinthine nystagmus is slow in one direction and quick in the opposite direction. The eye movements following cerebellar extirpation greatly outlast those of labyrinthine origin, and the labyrinthine movements may be superposed on those following cerebellar removal.

Removal of one or both occipital lobes of the cerebrum does not abolish labyrinthine nystagmus. True labyrinthine nystagmus has never been observed after complete decerebration, although the slow deviation of the eye persists. The slow component of nystagmus is of labyrinthine origin. The quick component is probably of cerebral origin.

The results suggest that the vestibular mechanism is connected far more closely with the phylogenetically older motor system (von Monakow) than with the phylogenetically newer system.

8 (617)

Experimental nephritis in guinea-pigs by subcutaneous injections of chromates.

By **W. OPHÜLS.**

[*From the Pathological Laboratory of Cooper Medical College and Stanford University.*]

After having determined that 1 centigram of bichromate of potash is very nearly a lethal dose for guinea pigs of from 500–750 gm., forty guinea pigs were used in an effort to produce, if possible, lasting anatomic lesions in the kidneys by repeated injections. Great difficulty was encountered in continuing larger doses on account of the extensive necroses produced at the site of injection. In the end it was found that a $\frac{1}{2}$–$\frac{1}{5}$ per cent. solution of chromate of potash to which an equal amount of carbonate of soda had been added was most satisfactory, although still quite irritating. As our experience has taught us that sublethal doses are most effective in experiments of this character, injections of one or one half centigram were used in one half of the experiments and the doses crowded as closely as the animals would tolerate; in other series smaller doses down to a quarter of a milligram were employed and continued for long periods (in one case for nearly two years). The immediate effect of the injection of large doses in the guinea pig is the production of an albuminuria which is usually quite limited in amount and the appearance in the sediment of desquamated cells from the uriniferous tubules, much more rarely of casts. The kidneys in the acute intoxication

are very markedly hyperemic, there is more or less fatty degeneration and well marked necrosis and desquamation of the epithelium, later cast formation also occurs. The glomeruli are hyperemic, do not show any distinct histologic lesions. Hemorrhages from them were not observed. The lesions are hardly severe enough to account for the early death of the animals. I have become rather strongly persuaded that the chromates cause death in guinea pigs not primarily by their action upon the kidneys, but in a different way, although nothing definite could be ascertained in this regard.

Results with Injections of One Half Centigram.—Several guinea pigs died from the first or second dose. Four lived from five months to 15½ months. The immediate reaction from each dose was well marked and it was impossible to give the doses very frequently; the highest was 12 doses in five months. One of these animals receiving five doses in seven months showed merely cystic dilation of some glomeruli similar to that which was observed in controls;[1] in one, apart from well marked epithelial lesions, there was a slight diffuse interstitial process; the other two showed definite small areas of collapse of degenerated tubules but with very little evidence of new formation of connective tissue between them.

Results with Injections of One Quarter of a Centigram.—Few animals died after the first injections; the immediate reaction on the part of the kidneys in most instances was very slight and the doses could be repeated more frequently. Some of these animals lived for one year or more, one for nearly two years. All showed more or less well marked epithelial lesions with formation of casts, very few interstitial lesions of a character which was not encountered in the controls also. In these few there were small areas of collapse of degenerated tubules and very little new formed connective tissue between them.

Experiments with smaller doses even when very frequently repeated and continued for long periods were entirely negative so far as the kidneys were concerned.

Hearts and bloodvessels remained normal in all animals.

The conclusion seems justified that it is impossible to produce

[1] See Ophüls, "Occurrence of spontaneous lesions in kidneys and livers of rabbits and guinea pigs," *Proc. Soc. for Exp. Biol. and Medicine*, 1911, viii, 75.

severe lasting renal lesions in guinea pigs with chromates, probably because in these animals the chromates are too toxic in a general way and too slightly effective on the kidneys locally.

9 (618)

Experimental nephritis in rabbits by subcutaneous injections of chromates.

By **W. OPHÜLS.**

[*From the Pathological Laboratory of Cooper Medical College and Stanford University.*]

Rabbits are relatively more susceptible to the action of chromates than guinea pigs. Animals of from 2,000–2,500 gm. in weight sometimes die after the injection of two centigrams of bichromate of potash. The acute renal lesions produced by large sublethal doses are much more marked than those found in guinea pigs under similar conditions. There is marked albuminuria, much degeneration, necrosis and desquamation of the epithelium and abundant formation of casts of different kinds. The extreme lesions which may develop after a while as a result of crowding of large doses were described by me in 1908,[1] but they do not necessarily follow even repeated administration of large doses.

Fifty animals were experimented upon. The dosage varied between two centigrams and two milligrams. Some of the animals were kept alive for a year or more. So far as epithelial lesions are concerned the experiences are similar to those in guinea pigs, except that the epithelial lesions became more severe and seemed to continue longer after the last injection. Very marked interstitial lesions were observed at times, but they resemble those observed spontaneously[2] so closely and occurred so irregularly, sometimes soon, sometimes late after the administration of various doses and sometimes not at all, that any definite conclusion of their relation to the injections could not be arrived at.

Hearts and bloodvessels remained normal in all animals.

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[1] Ophüls, " Some interesting points in regard to experimental chronic nephritis," *Journ. Med. Res.*, 1908, xviii, 49.

[2] Ophüls, *l. c.*

10 (619)

A dominant sex-limited character.

By **T. H. MORGAN.**

[*From the Department of Zoölogy, Columbia University.*]

A new mutant of the fruit fly, *Drosophila*, characterized by abnormal arrangement of the black bands on the abdomen, proves to be dominant to the normal arrangement of the bands, and since the factor for the character is coupled with femaleness it may be assumed to be contained in the *X*-chromosome. Abnormal ♀ by normal ♂ gives abnormal males and females. These inbred produce in the next, or F_2, generation 50 per cent. abnormal ♀, 25 per cent. abnormal ♂ and 25 per cent. normal ♂. No normal females appear in this generation. Thus the *normal character* is sex-limited in relation to the abnormal.

The reciprocal cross, viz., normal ♀ by abnormal ♂ gives abnormal females and normal males. These inbred produce in the next, or F_2, generation, 25 per cent. abnormal ♀, 25 per cent. normal ♀, 25 per cent. normal ♂, and 25 per cent. abnormal ♂[1]. The explanation is as follows:

Abnormal ♀	= Ab. *X* − Ab. *X*.		
Normal ♂	= N. *X* − ——.		
F_1 ♀	Ab. *X* N. *X*.		
♂	Ab. *X* ——		
F_2	Ab. *X* Ab. *X*	= Ab. ♀.	
	Ab. *X* N. *X*	= Ab. ♀.	
	Ab. *X*	= Ab. ♂.	
	N. *X*	= N. ♂.	

It will be seen that the abnormal factor is contained in *X*, hence sex-limited inheritance. The explanation of the reciprocal cross will be clear from this example.

Five other cases of sex-limited inheritance have been found in *Drosophila*, viz., miniature wings, rudimentary wings, black color, bright red eye and orange eye. All of these are recessive characters, and *ex hypothesi* are also present or absent from *X*. By crossing a red-eyed, abnormal type with a white-eyed, normal

[1] Since the abnormal character overlaps the normal some difficulty is found in classifying the F_2 generation.

type two points are established: first, that the same chromosome *X* may carry both a recessive (absence) and a dominant character at the same time, and second, that a strong "association" or coupling of characters exists. Thus, when an abnormal red-eyed ♀ is paired with a normal white-eyed male the offspring are abnormal red-eyed males and females. These inbred have given in the second generation:

Abnormal red	♀	18
Abnormal red	♂	6
Abnormal white	♂	0
Normal red	♀	18
Normal red	♂	6
Normal white	♂	14

A strong tendency for the grandparental combination to reappear in the F_2 generation is manifest.

The reciprocal cross, viz., abnormal red-eyed ♂ by normal white-eyed ♀ gives abnormal red-eyed females and normal white-eyed males. These inbred have produced:

Normal white	♀	47
Normal white	♂	55
Abnormal white	♀	0
Abnormal white	♂	4
Normal red	♀	34
Normal red	♂	19
Abnormal red	♀	41
Abnormal red	♂	48

Here also the normal and white combination reappear, while the abnormal and white are scarcely represented.

Trypanosomiasis in monkeys (Macacus rhesus) in captivity.

By **B. T. Terry.**

[*From the Laboratories of the Rockefeller Institute for Medical Research, New York City.*]

In the blood of an experimental monkey (*Macacus rhesus*), Dr. Richard Lamar found on October 28, 1911, an actively motile trypanosome. This discovery led to my examining the blood of all of the monkeys at the Rockefeller Institute.

In examining 130 monkeys, 28 were found infected with trypanosomes. The infected monkeys had been used for experiments between July 17 and October 6. Six monkeys used on or before July 17 were negative, and 80 others, some normal, the rest used after October 6, were also negative.

With but one exception, all of the monkeys examined belonged to the *Macacus rhesus* species.

The trypanosomes found in the 28 monkeys were apparently of the same kind. They have been successfully inoculated into one monkey (*Macacus rhesus*), six mice, two rats, one guinea pig, and one young rabbit. In none of these animals has a rich infection been seen. The two rats were infected on the 8th day, the six mice between the 9th and the 52d day, the guinea pig and rabbit on the 16th day, and the monkey between the 16th and the 24th day. The trypanosomes do not appear to be very pathogenic.

The micronucleus is usually at the extreme posterior end of the parasite, is strikingly large, measures 1μ or more in diameter, and often projects on either side of the parasite. The nucleus is oval, measures 1⅔ to 2⅔μ in its long diameter, and is situated near the juncture of the anterior ⅓ and the posterior ⅔ of the body. The flagellum is very long, the free part measuring 10 to 12⅓μ. The entire length of the parasites thus far measured has varied between 25 and 28μ, the breadth between 2 and 2¼μ.

If the future shows that the trypanosomes here described

belong to a new species, I propose for them the name *Trypanosoma rhesii*.

2 (621)

On the question of immunization against transplantable cancer by injection of an animal's own tissues.

By **R. A. LAMBERT.**

[*From the Department of Pathology, College of Physicians and Surgeons, Columbia University.*]

That the injection of a suitable quantity of homologous tissue induces in susceptible mice a certain degree of resistance to the inoculation of their transplantable cancers is a well-established fact. There appeared recently a paper by Woglom[1] in which experiments were described showing that autogenous tissue (spleen) injected subcutaneously was also capable of inducing this immunity. Woglom's work has been questioned by Apolant[2] who repeated the experiments with negative results. Apolant maintains further that the spleen of a mouse does not afford sufficient tissue for immunization, and that the question as to the possibility of immunizing with an animal's own tissues is still an open one.

In the experiments herewith reported, blood has been used as the immunizing agent. The quantity of defibrinated blood necessary for immunizing young mice was shown by Bashford to be about .3 c.c. In order to eliminate, however, the possibility of insufficient dosage, it was considered desirable to use at least .5 c.c. As Woglom stated in his paper, mice cannot be bled this amount plus the loss attending the bleeding without causing death. Interval bleedings from the jugulars were therefore resorted to. In nearly all of the experiments two bleedings only, on successive days, were necessary. About ten drops of blood in citrate solution were taken each time. The corpuscles were preserved in the ice box and injected subcutaneously on the day after the last bleeding. Ten mice were treated in this way; ten controls were injected with a similar quantity of homologous blood; ten normal mice were set aside as controls on the two

[1] *Jour. Exp. Med.*, January, 1910, p. 29.

[2] *Zeit. f. Immunitätsforschung*, July, 1911.

treated groups. The mice were all young and thrifty; average weight, 18 grams. The three series were inoculated with carcinoma ten days later. The record of the tumors resulting from these inoculations shows a definite immunity in the series treated with homologous blood, but practically no difference between the normal controls and those receiving injections of their own blood.

These findings, then, indicate that immunity against transplantable cancer in mice is, at least, not regularly induced by injections of an animal's own tissues. A larger series would probably be necessary to determine the existence of individual variations in the reaction of animals to such injections.[1]

3 (622)

Another case of sex-limited heredity in poultry.

By **C. B. DAVENPORT.**

[*From the Carnegie Institution of Washington.*]

To the four or five described cases of sex-limited heredity in poultry another is added.

The Jungle-fowl and its derivative, the Brown Leghorn, have the hackle and saddle feathered "laced" with red. The upper wing coverts of the cock are also red, forming the "wing bar." In the Dark Brahma, on the other hand, the red is not formed on the hackle and saddle and is nearly absent on the wing bar of the male, so that the red is replaced by white.

If, now, a male Dark Brahma be crossed with a female Brown Leghorn, or if the cross be made in the opposite direction, all sons are white-laced; but the wing bar is red. Thus, in these sons the white lacing is dominant but the white wing bar appears to be recessive. The most important point, however, is that the sons derived from the reciprocal crosses are practically indistinguishable.

With the daughters this is by no means the case. When the father is white-laced, the daughter is also; but if the father is red-

[1] A second set of experiments has been done which shows a slightly larger percentage of resistant animals among those immunized with autogenous tissue than among the normal controls. The difference, however, is not sufficiently striking to influence the conclusion drawn from the first experiments.

laced the daughter is likewise; *i. e.*, the daughter's lacing comes from the father's side of the house only.

The explanation is simple on the assumption that the lacing is linked with the sex-chromosome, following the scheme of transmission of the sex-chromosome as worked out by Stevens, Wilson and Morgan. While the male of poultry must have two somatic sex-chromosomes the female has only one. Therefore, while all sperm possess a sex-chromosome, only half of the eggs do. In the fertilized egg or zygote that has only one sex-chromosome, this is derived from the father and the zygote becomes a daughter. Hence the daughter "inherits" from the father only.

The hybrids have been bred together and a second generation has been obtained. When the hybrid male is mated to a white-laced hybrid female all the sons are white-laced while half the daughters are white-laced and half red-laced. But when the hybrid male is mated to a red-laced female half of the sons are white-laced and half red-laced while half of the daughters are white-laced and half red-laced as in the reciprocal mating. This result accords with the hypothesis.

4 (623)

The gastric and pancreatic secretions of the newborn.

By **ALFRED F. HESS.**

[*From the Department of Health, New York City.*]

I have been able by means of a simple duodenal catheter to obtain for the first time access to the duodenum in the living infant. This has enabled me to investigate the secretion of the upper part of the small intestine. This catheter is, in brief, merely a Nelaton soft rubber catheter No. 14 (F). That I actually do reach the intestine is proved by X ray photographs which I show.

The present report concerns solely newborn infants, which had never obtained any nourishment. I have found some interesting conditions not only as concerns intestinal secretions but also regarding the secretion of the gastric juice. It is noteworthy that there have been no previous investigations in this regard; in fact I have been able to find note of only one test of the gastric secretion of the newborn before it has been given food.

I found that in almost all cases hydrochloric acid is present in the stomach of these newborn infants whether they are examined one half hour or twenty hours after birth. The hydrochloric acid varies from ½ cu. cm. to about 7 cu. cm., and is not in direct proportion to the age of the child. This hydrochloric acid is not stimulated merely by the passage of the tube, for it was obtained within a minute or two after the catheter was introduced. It, therefore, must have another origin. I believe that it is a reflex due to the sucking of the tube. The longer the catheter is kept in the stomach the more juice is obtained; in one hour 14 cu. cm. was obtained; in an hour and 50 minutes 17 cu. cm. This gastric juice has an acidity on an average of about 50. Pepsin and rennet were also present in considerable amount.

These facts show that the infant at birth is prepared to digest food. The large amount of hydrochloric acid may be present in order to digest the high percentage of proteid of the colostrum and it may also serve the purpose of a bactericide, for we have found it to possess high bactericidal power.

The duodenum also was entered by means of the catheter. It can be entered almost as easily and quickly as the stomach in the newborn infant, and the contents aspirated. It was found that in the newborn infant there is very little to be aspirated from the duodenum compared to older infants. In spite of the fact that a large amount of hydrochloric acid is present, this does not seem to stimulate to a proportional degree the flow of the duodenal secretion. However, I have been able by this method to find protease, lipase, and amylase in the duodenum before any food was ingested, and, therefore, can state that the hydrochloric acid is sufficient hormone to stimulate these ferments. Amylase was found with least regularity. Frequently no secretion at all was obtained. In all about 35 tests were undertaken.

It is of special interest to note that bile was not found in these tests. It was expected that this would be quite otherwise, as the meconium is rich in bile, and we know that bile is found in the gall bladder of the fetus by the fourth month. I have not tested for the presence of the bile salts. Many other problems suggest themselves for investigation by this method; with some of these I am at present occupied.

5 (624)

Nitrogen and sodium chloride excretion in experimental uranium nephritis.[1]

By **HERMAN O. MOSENTHAL.**

[*From the Departments of Biological Chemistry and Medicine, College of Physicians and Surgeons, Columbia University, New York.*]

A series of experiments were performed upon dogs with the object of determining the effects on the output of nitrogen and sodium chloride in nephritis produced by the subcutaneous injection of uranium nitrate. These dogs were fed the usual standard diets, also diets containing much meat, as well as food to which considerable amounts of urea and sodium chloride had been added.

The results of these experiments appear to justify the following conclusions regarding uranium nephritis.

1. The nitrogen secreted by the small intestine, as determined by the Thiry fistula method, is somewhat diminished.

2. The fecal nitrogen remains approximately unchanged.

3. The urinary nitrogen is not diminished even when the diet demands an excretion of one gram of urinary nitrogen per kilo of body weight of the dog.

4. In some cases the urinary nitrogen is increased. The source of this excess of nitrogen is problematical. It may be due to protein destruction of the body tissues caused by the same toxic agent as the nephritis. If this be true, the "rest" nitrogen of the blood serum should be higher than normal. Experimental attempts have been made to ascertain the facts in this connection but have not been pushed far enough to warrant a definite statement.

5. The sodium chloride excretion in the urine keeps pace with the intake even when considerable quantities are added to the food.

6. The above statements have held true for dogs after the first as well as after several large single injections of the drug at long intervals. One animal was tested after he had received as many as seven injections.

[1] Under the auspices of the Edward N. Gibbs Memorial Prize Fund.

6 (625)

The seat of action in tetany after parathyroidectomy.

By **W. G. MacCallum.**

[*From the Department of Pathology of the College of Physicians and Surgeons, Columbia University.*]

Both the immediate cause and seat of tetany are still obscure. It has been suggested, though never proven, that a poison must circulate in the blood to cause the tetanic twitchings. It has also been suggested that these twitchings may result from a lack of calcium in the circulating fluids. It has been shown that tetany does not appear in a limb to which the nerves have been cut. It is conceivable that this is due to the fact that no impulses reach that limb from the spinal cord. The present experiments show that if the nerves be cut during tetany, their electrical excitability remains the same as that of the intact nerves on the opposite side. Further, it is shown that if the nerves be cut before the development of tetany, they become hyperexcitable to an extent which equals that of the intact nerve on the opposite side, although they are quite separate from the spinal cord. Since degeneration occurs within two or three days, such observations must be made with due regard to this fact. The peculiar character of this rise in the excitability is seen in the figures obtained for the cathode and anode opening shocks to which the nerves become especially excitable. In complete anemia or after the death of the animal there is a period of about one half hour during which this characteristic rise in the excitability of the nerve to the cathode and anode opening shocks appears. Nevertheless, the excitability of the nerves is, throughout this period, very much lowered and the curve is in no way comparable, therefore, to that in tetany.

Transfusion of the blood of an animal in tetany into the vessels of a normal animal has not succeeded in producing a characteristic tetany nor even a marked change in the excitability of the nerve.

This is probably partly due to inadequate technique and partly to the action of the normal dog's parathyroids. If the leg of a normal dog be isolated, with the exception of the nerves which

remain in intact connection with the spinal cord, and if the vessels be anastomosed with those of a dog in tetany, so that the peripheral portions of the normal leg are bathed with tetany blood, the nerves of that leg assume the characteristic hyperexcitability of tetany, and twitchings may even occur. Reëstablishment of the connection with the normal circulation brings back the excitability to normal.

From these experiments, it may be concluded that the hyperexcitability is peripheral and is dependent upon some change in the character of the blood. Experiments with curare, to determine whether or not this is an affection of the nerves alone, have not yet been completed, although one has the general impression that the excitability of the muscles themselves is increased over normal. The value of the experiments is thought to lie particularly in the opportunity which is offered for the study of tetany blood modified in various ways and used as the fluid for perfusing an isolated extremity.

7 (626)

Curves from a case of transient complete heart block, showing constantly varying ventricular complexes.

By **ALFRED E. COHN.**

[*From the First Medical Division, Mount Sinai Hospital, New York.*]

The patient from whom the curves were taken has been under observation since August, 1910. He was at that time suffering from cardiac decompensation. It was clear from the physical examination that he had a valvular defect. Combined arterial and venous curves were made. These showed that for short periods every impulse from the auricles was answered by a ventricular contraction, while at others the ventricular contractions responded to every second beat of the auricles. Curves were taken in February and March, 1911. These were volume curves from the jugular vein and radial artery and likewise curves of the cardiac action current, registered with an Edelmann string-galvanometer. At first the ventricles responded to every second

auricular beat, but somewhat later there was complete dissociation between the two pairs of cavities. Before the patient left the hospital, the relation between the auricles and ventricles was normal. Electric curves were taken on December 19, 1911, when the ventricles responded to every auricular contraction.

The curves which are of interest date from the period of complete auriculo-ventricular dissociation. Each ventricular complex is represented by *R*-, *S*-, and *T*-waves. They vary from each other in that, when the *R*- is large, the *S*-wave is small; and that when the *S*-wave is large, the *R*-wave is small. The *R*-wave gradually increases in size and then gradually diminishes, when the *S*-wave gradually increases in size and as gradually diminishes. If the apexes of succeeding *R*- and *S*-waves, *i. e.*, the significant wave in each complex were joined, a waved line would result. So diagrammatic a sequence as this did not occur frequently, but there was a tendency to approximate to this description. Sometimes the transition from complexes of one type to those of another was abrupt. The time between the complexes was almost equal, except at points of transition, when it was reduced.

It is concluded from the variation in the shape of the complexes, that they are responses to stimuli arising at levels in the heart varying from some supraventricular position to the apex. The explanation of the shortened time at points of transition is difficult. No hypothesis yet suggested is satisfactory. The fact that there was merely a temporary and not a permanent dissociation may be a significant factor, although the *P–R* interval associated with the transitions is not of uniform length. (*P* is the wave representing auricular systole.)

8 (627)

Further observations on the tolerance of gases by the circulatory apparatus.

By **J. P. ATKINSON** and **C. B. FITZPATRICK.**

[*From the Department of Health, City of New York.*]

In the *New York Medical Journal* of November 26 and at the New Haven meeting of December, 1910, we gave observations on the quantities of gas (air) tolerated and the apparent relation of

the adrenals to this tolerance. We have further determined that section of the cord between the fifth and sixth cervical vertebræ interferes with this tolerance of gas (air). After the removal of the adrenals from two dogs *carbon dioxide* was tolerated up to 942 c.c. in one case and 952 c.c. in the other, in 1 hour and 23 minutes.

Two dogs were injected with *nitrogen*.[1] One received 32 c.c. in six minutes with marked depressions; the animal lived. Panting occurred. The other received 272 c.c. within 22 minutes and died; panting was not noticed. The lungs were collapsed at autopsy.

The tolerance of *oxygen* was tested on two dogs. One received 150 c.c. slowly and survived. The other received 312 c.c. in 23½ minutes and died. During the oxygen experiments, panting occurred. The lungs were practically normal.

The tolerance of *hydrogen* was tested in two dogs. In one 184 c.c. in 8 c.c. volumes were injected in 13½ minutes. Each injection caused a moderate depression. 60 c.c. were given in 20 c.c. volumes in 3½ minutes, and 20 c.c. in 5 c.c. volumes. The large volumes caused great depression and almost death. In the other dog 90 c.c. were injected in 7 minutes in 8 c.c. volumes without harmful results.

Sulphuretted hydrogen was used to study elimination on two dogs. Three c.c. saturated H_2S water were injected into the femoral vein. The breath almost immediately blackened lead acetate. Twenty-five minutes after tying and clamping off the adrenals 3 c.c. more of saturated H_2S water was injected and a similar result was obtained. 100 c.c. of H_2S water introduced into the rectum did not give the reaction at the mouth in 25 minutes. In the other dog 30 c.c. of H_2S water were injected into the duodenum. In 8 minutes it was detected at the mouth.

[1] In every case morphin sulphate was given before etherization of the dog. Each dog was killed and autopsied after the experiment.

9 (628)

The destruction of adrenalin by spinal fluid.

By **S. J. MELTZER.**

[*From the Department of Physiology and Pharmacology of the Rockefeller Institute.*]

Soon after the discovery of the profound effect of adrenal extract upon blood-pressure the question arose as to the fate of this extract in the blood. The rise of the blood-pressure after an intravenous injection of adrenin passes off in a few minutes and none of the adrenin is found to persist in the blood, no matter how large the injected dose has been. The quite natural explanation of this phenomenon was, that the blood destroys adrenin. But Oliver and Schäfer found that in a mixture of adrenin and blood, even after standing for 22 hours, the adrenin remained unaffected. It has been confirmed since by several investigators, that neither blood nor serum is capable of destroying adrenal extract. I shall not discuss for the present the problem of the fate of adrenin in the body in general. I wish only to report the discovery of the fact that there is at least one body fluid which is capable of destroying adrenin and that is spinal fluid. The observation was made by mixing human spinal fluid with adrenalin. The spinal fluids were obtained in the first place from a number of cases of poliomyelitis of the Rockefeller Hospital and from two cases of tuberculous meningitis, obtained for me by Dr. Flexner. But this destructive action is not specific to these diseases. I found it to be possessed by spinal fluids from cases of resolving pneumonia, gastro-enteritis and eczema, obtained through the kindness of Dr. Wollstein. Evidently it is a physiologic property of normal spinal fluids, although there seems to be a difference in degree of action between some pathologic cases; for instance the spinal fluid from poliomyelitis seems to destroy adrenalin definitely more readily than that from tuberculous meningitis. *This fact might attain a practical significance.* The presence of adrenalin was studied by its dilating action upon the frog's pupil (the so-called Meltzer-Ehrmann reaction) and its action upon blood-pressure.

The tracings speak for themselves. The mixture of adrenalin with spinal fluid in proportion of 1:20 when kept on ice caused a considerable rise of blood-pressure by a dose of 0.5 c.c., while when this mixture was incubated for an hour in the thermostat at 37° C. even 4 times the dose caused no change in blood-pressure.

10 (629)

Glucuronic acid determination (Tollens) in duodenal obstruction.[1]

By JOHN WILLIAM DRAPER AND FREDERICK W. SCHLUTZ.

While the liver generally plays a subordinate part in the synthesis of glucuronic acid, it would seem from the experiments of Pohl (1), that upon the incorporation of chloral-hydrate or camphor, the conjugation of these substances with glucuronic acid does take place largely in that organ.

We have been interested in seeking a measure of the functional activity of the liver before and after experimental duodenal obstruction. By giving a dog camphor and determining the output of camphor-glucuronic acid, both before and after obstruction, we hoped to measure at least in a relative way any impairment in liver function which may follow this intestinal lesion.

The experiments were carried out on dogs—the operative portion under complete ether anesthesia. The animals were fed for fully a week on an exclusive meat diet in order to free the urine as much as possible from pentoses.

For the glucuronic acid determinations we employed one of the two methods described by C. Tollens (2, 3), viz., distillation of the glucuronic acid lacton with dilute hydrochloric acid, and precipitation of the resulting furfurol with phlorglucin. This method seems open to less objection that most of the other quantitative methods which have been proposed. In the hope of further determining the accuracy of our results, we are now experimenting with the CO_2 method described by Tollens.

[1] Studies from the Laboratory of the Department of Physiology, University of Minnesota, Minneapolis, and from the Laboratory of Surgical Chemistry and Physiology, Rochester, Minnesota.

The experiments here reported were carried out as follows: The glucuronic acid excreted during one or two 24-hour periods was determined and used as a normal. According to Tollens, the variations in the daily quantities of glucuronic acid are very slight, if the diet is uniform.

From 2 to 5 gm. of commercial camphor dissolved in olive oil were given subcutaneously and the camphor-glucuronic acid excretion in the urine determined for three to four 24-hour periods. The duodenum was then obstructed by cross section and infolding at a point just aboral to the greater pancreatic duct. From 12 to 24 hours after obstruction, determined by the first indication of marked clinical symptoms, the same dose of camphor was again given and the camphor-glucuronic output determined in 24-hour periods until death ensued.

Including a report made by Draper (4) on one dog, we have completed experiments on three dogs. The results, expressed in per cent. of recovered camphor-glucuronic acid, are as follows: From Dog No 30, before obstruction, 41.76 per cent. of a theoretical total was recovered. From Dog No. 124, 44.00 per cent., and from Dog No. 128, 43.47 per cent. After obstruction, from Dog No. 30 was recovered 27.19 per cent. of a theoretical total; from Dog No. 124, 19.29 per cent. and from Dog No. 128, 18.05 per cent. Somewhat less than one half the camphor given during the normal period was recovered in the urine as camphor-glucuronic acid. What becomes of the balance is a matter of conjecture. The results obtained by Schlutz (5) in an earlier study differ from the above but this can probably be accounted for by the inferiority and uncertainty of the polarimetric method. This was at the time considered the method of choice.

The histological examination of the livers, hearts, spleens and kidneys of the animals cited in this report was negative except for a moderate capillary dilatation in a few of the sections. The nitrogen metabolism remained unchanged in Nos. 30 and 124. It was not determined in No. 128.

The above results from their uniformity suggest accuracy for the method so far as it goes and the marked decrease after operation implies possible diminution of certain liver functions after duodenal obstruction.

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11 (630)

The convulsant effect of the removal of the heart upon frogs which had injections of morphin. A demonstration.

By **T. S. GITHENS** and **S. J. MELTZER.**

[*From the Department of Physiology and Pharmacology of the Rockefeller Institute.*]

Last April it was reported from this laboratory that (1) substances which are capable of causing a definite biological reaction in frogs cause the characteristic reaction also when injected after the removal of the heart, and (2) that some substances, like morphin and acid fuchsin, appear to be even more effective in cardiectomized than in normal frogs. The first phenomenon was explained by the theory that distribution in frogs deprived of the cardio-vascular circulation is accomplished by a peripheral mechanism, namely, by the tissue spaces, which present a connected system throughout the body. The second phenomenon was interpreted by the hypothesis that the fresh blood in the cardio-vascular mechanism continually antagonizes the convulsant action of such substances as morphin and acid fuchsin. The study of the last mentioned hypothesis and the underlying phenomenon has been hampered by the fact that the distribution by the peripheral mechanism is necessarily a slow one and since, at the warmer seasons, frogs survive cardiectomy only a short time, it happens that the animals die before the convulsant effect could make its appearance. We have therefore tried to study the hypothesis by the reversed method, that is, morphin injected first and the heart removed later. This was carried out in several series, the doses varying from 0.1 to 0.5 mg. of morphin per gram frog, and the intervals between the injection and the subsequent removal of the heart varying from a few minutes to 4 hours. We shall not enter

upon details: we shall merely state that the result was strikingly positive. While the morphin frogs, which were kept with hearts intact, remained normal, all the frogs which received proper doses of morphin and had their hearts removed at different intervals, developed tetanic convulsions, which in many cases had to be characterized as very violent. With doses of 0.25 mg. per gram frog, convulsions developed in practically every case, no matter how soon or how late the heart was removed. After intervals of 60 minutes and longer the result was positive practically with every dose between 0.2 and 0.5 mg. per gram frog. (The essential points were demonstrated before the Society.)

12 (631)

Intravascular foreign bodies.

By **C. C. GUTHRIE** and **A. H. RYAN.**

[*From the Laboratory of Physiology and Pharmacology, University of Pittsburgh.*]

Experiments were performed by introducing sterilized (1) untreated, (2) oiled silk, and (3) human hair into arteries and veins and observing the results. Twelve common carotid arteries and twelve external jugular veins were employed. The number of silk strands varied from the smallest single strands used in blood vessel suture to twenty-four such strands. Where a number were used they were threaded into suitably large needles and were not twisted. Cambric needles were used.

The experiments were performed by exposing the vessels of anesthetized dogs, transversely piercing the vessel with the needle as near the mid-line as possible, drawing the ligatures through and loosely tying the free ends together. Three weeks later specimens were taken and examined.

In no instance was there occlusion of the lumen nor was there any evidence to indicate that the vessels would have subsequently become occluded through thrombus formation. In general the ligatures were found dividing the lumen and coated with a substance closely resembling the intima in gross appearance.

In the case of one artery and one vein, an ordinary occluding ligature was first tied about the vessel, after which a transverse suture was introduced about one centimeter on each side of the ligature. The results in these cases did not materially differ from those observed on the non-occluded vessels. No marked differences were observed with different kinds or sizes of sutures.

Microscopical results, particularly as regards the character of the deposit on the foreign surfaces and the presence or absence of intimal covering, will be reported later.

13 (632)

On tumor-immunity in rats (with demonstrations).

By **RICHARD WEIL.**

[*From the Department of Experimental Therapeutics, Cornell University Medical School, New York City.*]

It is the intention of the present paper to summarize briefly experiments bearing on the problem of the acquired immunity of rats to the implantation of tumors. In rats, as indeed in practically all the other species of animals in which similar experiments have been practicable, it has been found that the absorption of an implanted tumor is succeeded by a period during which the animal is refractory to further implantations. The same refractory condition can be induced by the injection of tumor autolysates (Levin), or by the injection of suspensions of normal tissue, or even of blood. The relative specificity and effectiveness of these procedures vary in their details; the underlying principle has been thought to be the same throughout.

In a series of experiments with the Flexner-Jobling rat tumor, I have observed certain phenomena which seem in a measure to support the belief that the mechanism of immunity to the tumor is identical in its biological manifestations with that found in infectious disease, and is therefore mediated by immune substances carried by the serum. These observations may be summarized as follows:

1. Inoculation of the Flexner-Jobling adeno-carcinoma into normals rats is not followed by any macroscopic evidence of

inflammation or reaction, if the grafts are made from a small, non-necrotic tumor.

2. Rats specifically immunized to the Flexner-Jobling adenocarcinoma respond to an implantation by an intense local inflammatory reaction, often with necrosis.

3. Animals bearing necrotic tumors present this reaction on reinoculation.

4. After removal of the necrotic tumor, the animal still reacts to reimplantation by local inflammation.

These phenomena seem analogous to those instances of local anaphylaxis known as the "Arthus" phenomenon in rabbits, and to the local allergetic reactions described in human beings by Pirquet. Until the possibility of a reaction to a complicating infection of the tumor can be excluded (as suggested by Apolant of Yamanouchi's experiments), this interpretation must be considered provisional.

In the sarcomata of rats, it has not been possible to elicit these phenomena.

SCIENTIFIC PROCEEDINGS.

ABSTRACTS OF THE COMMUNICATIONS.

Forty seventh meeting.

The Laboratory of Natural History, College of the City of New York. February 21, 1912. President Morgan in the chair.

24 (633)

The fermentation of carbohydrates and other organic media by streptococci.

By **C. E. A. WINSLOW.**

[*From the Department of Public Health, American Museum of Natural History, New York.*]

The study of the fermentative powers of the streptococci has been carried out in England by merely noting the change of color in litmus media. In this country, Palmer and the writer, Broadhurst and Hilliard have used the more exact methods of titrating inoculated tubes and uninoculated controls, using phenolphthalein as an indicator and plotting the quantitative results obtained. The line of demarcation between fermenting and non-fermenting forms is drawn at the intermodal point between the peaks of the curve. A comparison of several hundred results obtained by English and American methods shows that the English method gives a uniformly higher proportion of fermenters, suggesting that a number of strains producing a very slight amount of acid and properly classed as non-fermenters, are recorded as positive by the English method.

A study of the correlations between action on different organic media shows that those substances tested may be arranged in a definite order of availability, such that a positive reaction in one medium usually implies that all those earlier in the series will be fermented, while failure to act on a given substance almost always implies that substances later in the series will not be fermented either. Among the streptococci dextrose comes first in order of availability, then the disaccharides, lactose and saccharose, and the glucoside, salicin (which easily yields a simple sugar). The

starch-like body inulin and the alcohol mannit come next and the trisaccharide raffinose is least available of all. This order corresponds to the size of the molecule, whereas in the *B. coli* group, the configuration of the molecule is the main thing, the aldehydic sugars being acted upon very readily, and the ketonic sugars less readily. The bacillus of the colon group which can utilize the ketonic disaccharide can almost always utilize the ketonic trisaccharide raffinose as well.

25 (634)

The comparative resistance of spores and vegetative cells of bacteria towards calcium hypochlorite.

By **C. M. HILLIARD.**

[*From the College of the City of New York.*]

At least three distinct grades of resistance to the disinfectant action of calcium hypochlorite may be recognized among the bacteria. They are respectively ordinary vegetative cells, acid-fast organisms, having a fatty composition, and the true spore cells. The present study is concerned only with the vegetative cell and the spore. Subsequent work will be done with the tubercle bacillus as representing the acid-fast group.

Pure cultures of *B. coli*, *B. prodigiosus*, *B. subtilis* (spore former) and *B. anthracis* (spore former) were grown in broth for a time sufficient for spores to appear in large numbers in the two latter cultures. A few drops were then transferred to dilution bottles, the initial number present was determined, and the calcium hypochlorite of known strength was added in carefully weighed amount. Agar plates were made at intervals and the reduction determined.

The results of the work to date may be summarized as follows:

1. The sterilizing action of calcium hypochlorite in water is very rapid at first, the maximum reduction being nearly complete in three hours.

2. Of the organisms studied, *B. subtilis* is most resistant, followed by *B. anthracis*, and then by the non-spore formers, *B. coli* and *B. prodigiosus*.

3. At least 1.5 parts of available chlorin to 1,000,000 parts

of water is necessary to get 99 per cent. reduction with a spore former in six hours, while 0.5 part to 1,000,000 is sufficient for this degree of reduction with non-spore formers.

4. Sixteen parts of available chlorin per million of water does not effect complete killing of *B. subtilis;* 1.5 parts brings about complete sterilization with *B. coli* and *B. prodigiosus*.

26 (635)

Studies on barium feeding.

By **C. L. ALSBERG** and **O. F. BLACK.**

[*From the Office of Drug Plant, Poisonous Plant, Physiological and Fermentation Investigations, Bureau of Plant Industry, Department of Agriculture, Washington, D. C.*]

Half-grown rats fed for some months on a mixed diet to which $BaSO_4$ was added remained in good health and did not store up measurable traces of barium in their tissues. However, when instead of a mixed diet one poor in calcium was fed, other conditions being the same, a few milligrams of barium were stored in the tissue of each animal. It is therefore evident that under special conditions even very insoluble substances may be absorbed to some extent.

27 (636)

The production of grafted and multiple embryos.

By **A. J. GOLDFARB.**

[*From the College of the City of New York.*]

After removing the fertilization membranes of sea urchin eggs (*Arbacea*) and allowing them to develop to the desired stage, the eggs were placed in an alkaline (NaOH) sea water and centrifuged in narrow bore tubes. In this way large numbers of eggs were agglutinated, and developed into double, triple, etc., blastulæ, gastrulæ and plutei. In ten to forty per cent. the eggs and blastomeres were more or less completely fused, forming giant blastulæ, composed of three, four or more eggs.

In the first group parallel development took place, resulting in double, triple, etc., embryos, many of which were subsequently separated by the antagonistic sweep of the cilia. In the second group, the eggs were more intimately united to form a common

blastocœle, common gut, or common body, within which the independent organs may or may not unite. When the embryos fuse slowly the contained organs appear to be antagonistic, for one is often absorbed completely, or the interaction results in a united but very atypic gut, skeleton, body, etc. The details of these changes are exceedingly interesting, but can be given only in the fuller publication.

28 (637)

The production of typical monstrosities by various means.

By **A. J. GOLDFARB.**

[*From the College of the City of New York.*]

Typical abnormalities have been produced by subjecting developing eggs to the action of certain salts, such as lithium chloride, and the implication, if not the conclusion has been made that a certain specificity obtained between these salts and the resulting abnormality. Herbst for example produced definite atypic gastrulæ, by subjecting sea urchin eggs to lithium chloride in sea water. Stockard produced definite atypic conditions of the eye and brain formation in *Fundulus*.

That the same results may be obtained in other ways seems to demonstrate that the extra-gastrulate condition of the sea urchin, for example, is due not so much to any specific action of the lithium chloride, as to a factor common to each of the following. Extra-gastrulate embryos were produced in fairly large numbers by such anesthetics as chlorotone and alcohol, by changing the concentration of the sea water in opposite directions, either by dilution or by concentration of the sea water, by the action of carbon dioxide and lastly various sugar solutions.

Similarly other well-defined atypic blastulæ, gastrulæ or plutei, though not always produced by each of these solutions, were found in many of them, thus giving color to the view that a disturbance once set up results in a typical reaction conditioned not so much by the nature of the disturbance as by the mechanism (the egg) involved.

29 (638)

The percentage of water in the brain of the dog-fish.

By **G. G. SCOTT.**

[*From the Department of Physiology, Columbia University, and Biological Laboratory of the U. S. Bureau of Fisheries, Woods Hole, Mass.*]

Donaldson ('10) has shown that in the albino rat between birth and maturity the percentage of water in the brain diminishes from 87.8 per cent. to 77.5 per cent. He calls attention to the fact that the human brain at birth contains a greater percentage of water than at maturity and from the investigations of Koch and Weisbach he obtains as the percentage of water in the human encephalon to be: Birth 88.3 per cent.; 2 yrs. 81.1 per cent.; 5 yrs. 79.2 per cent.; 25 yrs. 77.0 per cent. He concludes that probably in all mammals we will find the same range in percentage of water, that the loss in water occurs in the same manner but that the time required for each successive step is determined by the intensity of the growth process characteristic of each period. The present writer determined the percentage of water of the brains of 17 spiny dog-fish (*Squalus acanthias*) and 97 smooth dog-fish (*Mustelus canis*). The smooth dog-fish ranged in size from very small to large. There is no such reduction in the percentage of water as found by Donaldson and others in the case of the mammalian brain. The average percentage of water in all the *Mustelus* brains examined was 78.5 per cent. There was very little difference between this and that obtained for the very young or the old. There is an indication of a slight fall of about 2–3 per cent. between birth and maturity. The great reduction (*i. e.*, about 7 per cent.) occurs in mammals during the period when the central nervous system is growing most rapidly. Both the rat and man during this short post-birth period pass from a helpless state to one of activity. The rat is born helpless and blind, etc. The dog-fish is an active free-swimming organism at birth. The present writer would conclude then that the differences in reduction of water in the two cases is that the nervous (and body) changes which occur in the mammal are post-embryonic and extra-utero. In the dog-fish they take place in utero. Determinations from brains of late embryonic stages can only settle this hypothesis.

30 (639)

The advantage for certain experiments *in vitro* of suspending trypanosomes in serum.

By **B. T. TERRY.**

[*From the Laboratories of the Rockefeller Institute for Medical Research, New York, and from the George Speyer House, Frankfort a/M, Germany.*]

The amount of trypanocidal substance contained in a given solution is sometimes estimated *in vitro* by determining the degree to which the solution may be diluted before it ceases to immobilize the parasites.

For want of a better diluent, trypanosomes have usually been suspended in physiological salt solution (with or without the addition of citrate), although salt solution not infrequently immobilized the parasites in 30 to 60 minutes.

In 1910, the writer made observations which caused him to substitute serum for the salt solution he had previously employed in suspending *T. brucei.* Serum (when not bound by the medicament under investigation) had the following advantages:

1. The motility of the control parasites was greatly prolonged. This enabled the observations to be continued over a longer time.

2. The motility of the control parasites was accelerated. This enhanced the delicacy of the tests *in vitro* by rendering more striking the contrast between the poisoned and the non-poisoned trypanosomes.

It was found, moreover, that poisons not infrequently immobilized more quickly trypanosomes suspended in serum than they did those suspended in salt solution. This also seemed to give serum a slight advantage over salt solution as a medium in which to suspend trypanosomes.

Rabbit, ox, horse, goat, sheep, pig, chicken, rat, and mouse sera were tested and were found to be efficient in prolonging the motility of trypanosomes.

It soon became easy to keep on hand a large supply of serum, for experiment showed that cattle serum, filtered through a Berkefeld filter, bottled aseptically, and preserved in the ice-box, retained its activity for many months.

That the motility of trypanosomes is preserved longer in serum than in salt solution was noted years ago and has recently been emphasized by Schern,[1] but the writer is not aware that anyone has previously recommended suspending trypanosomes in serum for experiments *in vitro*.

31 (640)

The action of atoxyl.

By **B. T. TERRY.**

[*From the Laboratories of the Rockefeller Institute for Medical Research, New York.*

The action of atoxyl is paradoxical. *In vivo* it is effective against certain parasites, *in vitro* it has little or no action. Ehrlich believes that the medicament must be reduced in the body before it becomes active. This view has been strengthened by Levaditi and Yamanouchi who have shown that emulsions of liver, muscle, and lung, when incubated with atoxyl, transform this medicament into a toxic substance. Levaditi apparently believes that the transforming agent is in the liver and other organs, while Yamanouchi concludes that it is in the red blood cells only. My results confirm much of the experimental work of Levaditi and Yamanouchi, but lead to a conclusion that, in its entirety, is apparently held by neither of these investigators.

In my experiments, both liver and blood when incubated with atoxyl (10 per cent.) at 37 degrees for 3 hours, transformed this medicament into a toxic substance.

The transforming agent in liver had characteristics, however, which in some respects were quite different from those of the active agent in blood.

The active agent in liver was soluble in salt solution, was filterable through collodium, and was quite resistant. Liver emulsion ground with sand in a mortar, or heated to 100 degrees for 10 m., lost little or none of its activity. The addition of blood to liver emulsion before incubation with atoxyl increased its activity, but liver emulsion washed *thoroughly* to free it of red blood corpuscles was inactive, probably because of the solubility of the transforming agent.

From the blood the active agent was apparently not extract-

[1] "Arbeiten aus dem kaiserlichen Gesundheitsamte," Berlin, 1911, xxxviii, 338.

able by salt solution. Moreover, it was very easily destroyed, soon losing all or nearly all of its activity if the blood was laked (*e. g.*, by the addition of distilled water or saponin, by prolonged shaking at 37 degrees, or by grinding in a mortar with sand and salt solution, or with sand and serum). It was almost completely destroyed by heating to 100 degrees for 10 minutes.

Conclusion.—Atoxyl is probably transformed into a trypanocidal substance in the living body both by the blood and by the liver (other organs were not tested). In tests *in vitro* the transforming agent in liver may be readily distinguished from the active agent in blood.

32 (641)

Parturient paresis and eclampsia. Similarities between these two diseases.

By **DANIEL J. HEALY** and **JOSEPH H. KASTLE.**

[*From the Laboratory of the Kentucky Agricultural Experiment Station.*]

In June, 1907, the attention of one of us (Healy) was called by Dr. M. A. Scovell, Director of the Kentucky Agricultural Experiment Station, to parturient paresis in the dairy cow. Dr. Scovell's intention was to have, if possible, the etiology cleared up.

It proved impossible to take up the problem until one year ago, and as our studies progressed, the similarity between parturient paresis and eclampsia became more and more evident. They are both intoxications which occur suddenly just before, during or immediately after labor. They are characterized by the same clinical features, namely, suddenness of onset, loss of consciousness, coma and similar febrile conditions. In both, the urinalyses are the most important clinical features, and the urinalyses in these two conditions are similar, namely, a disturbance of the nitrogen distribution among the compounds containing nitrogen, an increase of the ammonia excreted, the presence of albumen, and microscopically the presence of hyaline, granular and epithelial casts and blood cells.

The finer pathological changes occurring in parturient paresis have not been established, and as none of our cases died, we have not had the opportunity of studying these changes. However, we

have had ample opportunity to study the finer pathological changes in three guinea pigs which died in five, six and seven days under the influence of the toxin of parturient paresis. We have also observed these changes in another guinea pig, which received a smaller dose of the toxin, the dose not being sufficient to cause death in ten days and therefore he was chloroformed. The pathological findings in these guinea pigs correspond in every way to the characteristic lesions of eclampsia, namely, there was hemorrhagic necrosis of the liver, acute parenchymatous nephritis with interstitial hemorrhages, degeneration of the cells of the adrenal cortex, with interstitial hemorrhages, and destruction of the medullary portion. These experiments were fully controlled in every detail by means of guinea pigs injected with normal salt solution, fresh milk, fresh normal colostrum and fresh, normal, cow's urine. The control pigs all remaining alive and well at the present time, with the exception of the normal urine pig, which was chloroformed at the end of seven days and on post mortem found normal.

The modern treatment of parturient paresis is most remarkable in its result. The mortality has been reduced from 60 per cent. to less than 1 per cent. It was introduced by J. Schmidt, of Kolding, Denmark, and was based upon the theory that the disease was due to bacterial invasion of the udder. The treatment consists of acute dilatation of the udder by means of oxygen or sterile air.

That the disease is due to a toxin elaborated in the udder as the result of its own metabolism preceding normal milk production, there can be no reasonable doubt, and that the success of the modern treatment is due to preventing, by means of pressure, the absorption of this toxin, seems most highly probable.

We are of the opinion that eclampsia is due to a similar toxin, elaborated by the breast in a similar manner, and would strongly recommend, as the most promising treatment, dilatation of the breasts with oxygen or sterile air, or forcible compression of them by means of a properly applied bandage, after they have been emptied by means of the breast pump, and at the same time using whatever medical measures may be indicated.

We, ourselves, shall thoroughly test this method of treatment as soon as the opportunity occurs.

33 (642)

The toxic character of the colostrum in parturient paresis.

By JOSEPH H. KASTLE and DANIEL J. HEALY.

[*From the Laboratory of the Kentucky Agricultural Experiment Station.*]

Parturient paresis is preëminently a disease of plethoric heavy milking breeds of cows, and of those individuals which give the greatest yield of milk. Among the prime and immediate causes of the disease are parturition, a permanent or transient plethoric condition of the blood vessels, with corresponding increase of pressure on the nerve centers of the brain. The phenomenal trophic and secreting activity of the udder of the heavy milker and intense physiological activity of the mammary glands resulting in the sudden rise and absorption into the circulation of leucomaines or toxic alkaloids of the cells of the mammæ. These according to Law[1] are the principal causes operating to bring on an attack of this disease. In the present state of our knowledge it is of little moment whether we call the substances, other than milk, resulting from the sudden disintegrative changes in the udder at or about the time of parturition, leucomaines, alkaloids, toxins, or what not. It seems reasonably certain, however, that there is no gland of the size and physiological activity of the udder of a heavy milking cow, but what must contribute very largely and sometimes malignantly to the internal secretions of the animal.

The question, therefore, immediately before us in the study of parturient paresis and of eclampsia in the woman is to determine experimentally whether the udder and the breast, respectively, do under these acutely toxic conditions actually secrete poisonous substances, which if not quickly eliminated or prevented from entering the circulation might be held responsible for these diseases.

It therefore occurred to one of us, Kastle,[2] to test the conduct

[1] "Text-book of Veterinary Medicine," 2d ed. (1905), Vol. 3, pp. 301–317 Ithaca, N. Y.

[2] On the day after our three papers on parturient paresis and eclampsia were mailed to the editor of the *Journal of Infectious Diseases*, Chicago, viz., on February 13, 1912, Dr. Surface called our attention to an abstract by Helfer of a paper by Hoyois in the *Berliner Tierarztliche Wochenschrift*, October 5, 1911, No. 40, pp. 727–

of the first fresh colostrum of the cow obtained during an attack of parturient paresis, upon the lower animals. We have not yet been able to obtain a case of eclampsia. Accordingly eighteen experiments have been carried out on guinea pigs. These experiments have included a few other substances besides the first colostrum of a cow suffering from parturient paresis, by way of comparison, and these of course serve as controls on our other results. Up to the present, therefore, the following substances have been tested on the guinea pig, and in all instances were given intraperitoneally, by hypodermic injection, using 10 c.c. or in some cases less, viz., the first, fresh colostrum of the normal cow, fresh milk from a high-class dairy herd, the first urine of a cow suffering from parturient paresis, the urine of a healthy cow, normal salt solution, and aqueous solutions of the residue from colostrum and milk left after precipitating the same with dilute acetic acid and finally the first, fresh colostrum of a cow, obtained during an attack of parturient paresis. Briefly the following results have been obtained.

The normal salt solution, the urine of the healthy cow, and the fresh milk from the healthy herd produce no bad effects when injected intraperitoneally into guinea pigs. The first fresh colostrum of the normal cow produced diarrhea in the guinea pigs from which they speedily recovered and these pigs are now alive and well. The dried residue obtained by evaporating the filtrates from normal colostrum after precipitating with dilute acetic acid and neutralizing produced no effect.

Three female guinea pigs received 10 c.c. each, of the first, fresh colostrum of a cow in an attack of parturient paresis. (1) The whole colostrum, (2) skimmed colostrum, (3) colostrum cream. Pig 1 had no diarrhea, but died on the sixth day. On post-mortem this pig showed acute parenchymatous nephritis with interstitial hemorrhages, acute parenchymatous hepatitis with interstitial hemorrhages, acute degeneration of the cells of the adrenal cortex with complete destruction of the medullary

728, the original of which appeared in the *Annales de Med. Vet. de Bruxelles*, July, Aug., Sept., 1910, in which according to Hoyois the colostrum in cases of parturient paresis, on intraperitoneal injection in doses of 10 to 20 grams, caused paralyzing symptoms in rabbits and guinea pigs, with subsequent death at the end of seven to twelve days.

cells, and interstitial hemorrhages. No evidence of tuberculosis. Cultures from the liver, kidney and spleen showed no micro-organisms. No peritonitis. The pig was not pregnant.

Pig 2 seemed well after the injection; had no diarrhea. Died at the end of five days. On post-mortem showed acute parenchymatous nephritis, with interstitial hemorrhage, acute parenchymatous hepatitis, with interstitial hemorrhage, and marked peripheral necrosis. Acute degeneration of the cells of the adrenal cortex, with complete destruction of the medullary cells and interstitial hemorrhages. No evidence of tuberculosis. The pig was in the very early stages of pregnancy. Cultures from the liver, spleen and kidneys negative. No peritonitis.

Pig 3 seemed well after injection and had no diarrhea. Aborted during first twelve hours after injection, fetus five and five tenths cm. in length. Died in six days. The post-mortem showed acute parenchymatous nephritis, with interstitial hemorrahges; acute parenchymatous hepatitis, with areas of complete necrosis and interstitial hemorrhages. Some degeneration of the cells of the adrenal cortex with complete destruction of the medullary portion, and interstitial hemorrhages. Acute lobar pneumonia of the left lung. No tuberculosis. Pig no longer pregnant. Cultures from the liver, kidney and spleen negative. Cultures from the pneumonic lung contained a diplococcus. No peritonitis except over upper and anterior surfaces of the liver.

A healthy male guinea pig received 10 c.c. of the first, fresh, clear urine of a cow ill with parturient paresis. This pig showed no discomfort and no diarrhea. It developed a very marked diuresis however, passing at least 200 c.c. of urine in 24 hours. This urine contained a small amount of albumin and no sugar. This pig recovered from the diuresis and seemed well, and was chloroformed on the 13th day. On post-mortem this pig showed parenchymatous nephritis with some interstitial hemorrhage, a rather extensive necrosis of the liver cells, but without hemorrhage, and also localized areas of necrosis in the adrenals.

It is evident from these results that normal salt solution, fresh milk, and the urine of a healthy cow cause no disturbances in healthy guinea pigs. The colostrum of the normal cow invariably produced a diarrhea when injected into the peritoneal cavity of

healthy guinea pigs. In this connection it is of interest to note that it has long been known that human colostrum acts as a mild cathartic on the suckling (Williams, " Obstetrics," 1908, 351–352). Otherwise no bad effects followed the administration of the normal colostrum of the cow to healthy guinea pigs. It will be seen from our results that death invariably resulted in guinea pigs from the intraperitoneal injection of the first, fresh colostrum of a cow in an attack of parturient paresis, and that the post-mortem examination of the organs of pigs that had died from this cause showed the same pathologic degenerations and changes that are shown by the organs of women who have died of eclampsia. Unfortunately but little is known regarding the micropathology of the cow in parturient paresis. We have shown, however, that cows recovering from an attack of this disease invariably show a nephritis which may, as the result of repeated attacks, become chronic.

Our results with the colostrum of a cow suffering from parturient paresis certainly go to show the presence therein of some substance or substances toxic to guinea pigs, and certainly point to the udder and the mammary glands as the place of origin of the toxins or internal secretions producing parturient paresis and eclampsia respectively. The fact that the urine of the cow with parturient paresis causes such a profound diuresis in the guinea pig, points to the presence of toxic substances even in the urine of animals so affected. A conclusion which is sustained by the results of the post-mortem examination on this particular case. We hope in the near future to attempt the isolation of the particular substance or substances in the colostrum or the udder, responsible for parturient paresis or at any rate, its more careful study and nearer identification. We would, therefore, reserve the right to continue these investigations along the lines indicated above with the object of throwing further light on the nature of the toxine contained in the colostrum of cows suffering from parturient paresis and also the possible occurrence of such a toxine in the colostrum of women suffering from eclampsia, and with the still further object of arriving, if possible, at the precise conditions under which these toxins are elaborated in the udder and mammary glands.

34 (643)

The internal secretion of the mammæ as a factor in the onset of labor.

By **DANIEL J. HEALY** and **JOSEPH H. KASTLE.**

[*From the Laboratory of the Kentucky Agricultural Experiment Station.*]

The importance of the internal secretions has come to be well recognized in modern physiology and medicine. Among other interesting observations in this field may be mentioned the fact that Miss Lane-Claypon and Starling (*Proc. Roy. Soc.*, 1906, B. 505) have shown that the stimulus to the hypertrophy and lacteal activity of the mammary glands, in pregnant animals, comes not from the ovaries, or the placenta or the uterus, but from the fetus itself. In connection with our studies on the toxic nature of the colostrum of the cow, ill with parturient paresis, we have succeeded in showing that the colostrum both of the normal cow and that of the cow ill with parturient paresis contain a substance, or substances, which have the power to bring on abortion in pregnant guinea pigs; and that neither normal salt solution (0.85 per cent. NaCl) nor the fresh milk of a healthy dairy herd have the power to bring on premature labor. It has also been shown that boiling for a short time does not destroy the power of the normal colostrum of the cow to accomplish premature labor in pregnant guinea pigs.

In this abstract of our paper on this subject we have only space for the details of one experiment, which are as follows:

Experiment 15.—A healthy guinea pig in the 5th to the 7th week of pregnancy received by intraperitoneal injection 10 c.c. of sterile, normal salt solution (0.85 per cent. NaCl). The injection caused no discomfort and at the end of five days she had not aborted. She then received by intraperitoneal injection 10 c.c. of fresh milk, from a healthy dairy herd. This was heated to 38° C. before the injection. The pig showed no discomfort and had not aborted at the end of four days. She then received, by intraperitoneal injection, 8 c.c. of the first, fresh, whole colostrum of a normal cow (second calf). This colostrum was heated to 38° C. before the injection. Following this last injection this

pig aborted in 60 hours, giving premature birth to two fetuses, each 6.5 cm. in length, and 60 hours after this she aborted a second time, giving premature birth to one fetus 7 cm. long.

It is evident from these results that the colostrum of the normal cow contains a substance, or substances, capable of causing a premature onset of labor in pregnant guinea pigs. This substance is also present in the colostrum of cows ill with parturient paresis. It resists boiling and is probably of the nature of a hormone. Our results bring to light a new and hitherto unrecognized correlation between the mammary glands and the uterus. According to Lane-Claypon and Starling the fetus through its internal secretions stimulates the hypertrophy and lacteal activity of the mammary gland. It is evident from our experiments that the internal secretions of the mammary gland stimulate the pregnant female to labor and the birth of the offspring.

35 (644)

Some vaso-reacting substances in blood serum.

By **J. P. ATKINSON** and **C. B. FITZPATRICK.**

[*From the Department of Health Laboratories, New York City.*]

These observations supplement our previous articles on serum digestive processes. All the reactions were obtained by the kymograph, the pressure in the carotid of the normal dog being recorded with a mercury manometer. The injections were made into the femoral vein. The normal sera (horse and rabbit) give a slight rise, whether freshly drawn or after standing for some weeks as far as we have observed.

The whole serum of a horse which had been injected with 800 c.c. of strong diphtheria toxin gave no reaction with the serum drawn the first three days after the injection. The serum of the 4th, 5th, and 6th day each gave depressions, when given in 8 c.c. volumes.

Another series of sera from the same horse, bled 6 weeks later gave well-marked depressions, with the sera drawn on the 3d, 4th, 5th, 6th, 7th, 8th and 9th day after the toxin injection.

Several specimens of refined antidiphtheria sera, some of which had given rise to rashes in humans, gave well-marked depressions.

These depressions are not caused by the injection of minute quantities of ammonium sulphate per se. A recent, whole, anti-pneumococcus serum, which produced rashes in humans, gave in a young dog well-marked depressions and after the total injection of 35 c.c. given in 7 c.c. volumes, there was a rise, 13 minutes after which the dog died. This depressor substance practically disappeared after four days standing in the ice-box.

We have noticed a similar rise after the injection of numerous 6 c.c. doses of beef extracts, each of which had produced marked depressions; we have however been unable to kill a dog with these injections. These observations would seem to indicate that the amount of depression per se within moderate limitations is not so important as the recoil or loss of recoil.

Three c.c. of nutrient peptone broth gave no depression. The blood sera of a rabbit, which had 5 days previously been injected with nutrient peptone broth gave splendid depressions in 6 c.c. volumes.

The blood sera of rabbits, which had been 5 days previously injected with heated, sterilized, cultures of the typhoid bacillus, pneumococcus, and streptococcus, gave remarkable depressions in 4 c.c. volumes.

The injection of 2½ c.c. of the serum of a rabbit which died 4½ hours after having received 1,000 m.l.d. of tetanus toxin intravenously, gave a decided depression.

The injection of 2½ c.c. of the serum of a rabbit which had 3 hours previously received 1,000 m.l.d. of diphtheria toxin, intravenously, gave a depression. The depressor substance of both of these sera practically disappeared, after standing 6 days in the ice-box.

Adrenalin chloride prevents the depression caused by the tetanus depressor serum.

Twelve m.l.d. and 15 m.l.d. of diphtheria toxin gave no reaction when injected into the dog intravenously, 3 c.c. of broth containing 210 m.l.d. of diphtheria toxin gave a well-marked depression when injected intravenously into a dog which had been sensitized 24 hours previously with 100 m.l.d. of diphtheria toxin.

The blood serum of rabbits injected with 3 m.l.d. of diphtheria and tetanus toxins, drawn 4 days after the injections, gave marked depressions.

Contaminated sera cause depressions.

An antimeningococcus serum, which had produced rashes in humans caused well-marked depressions in 6½ c.c. doses.

Serum of a diphtheria antitoxin horse, which was recovering from an attack of indigestion gave a slight depression. This serum was secured through the courtesy of Dr. Banzhaf.

These observations have extended over a period of 2½ years.

Note.—We have already reported depressions from the injection of the sera of tuberculous rabbits and from the injection of tuberculins as well as from the injection of the sera of animals inoculated subdurally with normal and hydrophobic brain tissue emulsion.

36 (645)

A study by the Meyer method of the effect of blood serum and certain inorganic salts on surviving arteries.

By **E. A. PARK** and **J. C. JANEWAY.**

[*Department of Medicine, Columbia University.*]

The method employed is an adaptation of the Meyer ox carotid method. Instead of strips from the carotid, rings from the mesenteric or hepatic arteries of the ox strung in pairs were used, and from the coronary arteries as controls. Adrenalin, even in every dilute solution, constricts the former, while it causes the coronary to dilate, whether it be added to Ringer-Locke fluid, or to the ox blood serum. This method, then, based on the contrary effects produced by adrenalin on two kinds of arteries, each possessing a different reactive property to adrenalin, should be ideal for the detection of adrenalin and the separation of it from the confusion with other substances in the blood serum exerting a constrictor or dilator action. Ox blood serum as opposed to adrenalin produces a constriction of both coronary and mesenteric or hepatic arteries. Thus it essentially differs in its action from adrenalin. There is, then, so far as surviving arteries are concerned, a vasoconstrictor property of ox blood serum, not to be explained by the presence of adrenalin.

The constriction produced by ox blood serum on ox arteries

occurs abruptly after a latent period of only a few seconds and is comparable in its intensity to that produced by adrenalin on the mesenteric or hepatic arteries. The duration is at least four hours, the limit of our means for recording it. Passing oxygen through it weakens this constricting property, as does time, *i. e.*, allowing it to stand one or two days at room temperature. Adrenalin added to blood serum even at the height of a contraction further increases it in the case of hepatic and mesenteric arteries, but produces an especially marked relaxation in the case of the coronary artery. Adrenalin added to fresh ox blood to make a proportion of one to 800,000, we have identified thirty-six hours later; and when added to make a proportion of one to 100,000 after seven hours' oxygenation under a pressure of more than 100 millimeters mercury at incubator temperature.

Sodium chloride in dilution less than .01 produces a marked constriction of the above-named arteries as compared with Ringer-Locke fluid. The latent period is longer than that of adrenalin or ox blood serum, the ascent more gradual; moreover the height of the curve seems to vary inversely to the sodium chloride water ratio to a point .005, below which we have not investigated. At .013 sodium chloride the strips of artery apparently die. The relations of calcium and potassium to tonus have not been taken up yet. Barium chloride produces a vasoconstriction which exceeds that produced by adrenalin, or, so far as our experience goes, any other substance. The curve produced by it tends to be irregular, frequently assuming a staircase character.

37 (646)

The influence of the sugar concentration of the blood on the protein metabolism in phlorhizin diabetes.

By **A. I. RINGER.**

[*From the Department of Physiological Chemistry of the University of Pennsylvania.*]

According to Rubner, the protein metabolism of a normal starving animal is composed of two fractions:

I. Wear and tear quota.

II. Dynamogenetic quota.

The first fraction represents the protein metabolized in the life processes of the cells. The second fraction represents the protein burnt for the purpose of maintaining the temperature of the body.

Landergren, however, presents considerable evidence to show that the "dynamogenetic quota" of Rubner is really the result of two distinct processes:

I. The protein that is metabolized for the increased production of sugar, in cases where sugar is absent from the diet and the glycogen supply becomes low.

II. For the maintenance of body temperature.

It is a well-known fact, that in phlorhizin diabetes, the protein catabolism rises enormously; in some cases as high as five times the starvation requirements. Because of the renal origin of the glycosuria, there is a constant tendency for the concentration of the sugar in the blood to fall. The following experiment was performed in order to test what part, if any, the concentration of the sugar in the blood plays in the regulation of the protein metabolism.

A dog was phlorhizinized in the usual manner, and the D : N ratio established. Seventy-five grams of glucose dissolved in water and divided into six doses were given *per os* on the fourth day of the glycosuria. 150 grams were given on the sixth day.

The results are here tabulated:

Dog No. 11.

Date.	Period.	Weight.	Total N.	Total Sugar.	D : N.	Remarks.
February, 1912						
15"	III.	17.53	14.40	52.08	3.62	
16	IV.	17.24	9.32	103.10	11.06	75 gm. of glucose given per os.
17	V.	16.86	14.00	50.95	3.64	
18	VI.	16.60	7.18	127.17	17.71	150 gm. of glucose given per os.
19	VII.	16.25	7.78	56.29	7.23	
20	Animal died under anesthesia, while a sample of blood was being withdrawn from the carotid artery.					

From the D : N ratio on the third and fifth days, we may assume that the phlorhizin intoxication was complete and that the protein burnt on the fourth, sixth and seventh days yielded 3.6 gm. of glucose for every gram of nitrogen.

The amount of glucose eliminated on the fourth day was 103.1 gm. By subtracting 33.55 gm., which originated from the protein (9.32 × 3.6), we find that 69.5 gm. of the 75 gm. of glucose fed, were eliminated unburnt. By applying similar calculations to the results obtained on the sixth and seventh days, we find that the protein metabolized during the sixth day yielded (7.18 × 3.6) 23.85 gm. of glucose, and during the seventh day (7.78 × 3.6) 28.01 gm. The total amount of glucose eliminated during these two days was 183.46 gm. By subtracting the glucose that was derived from the protein, we find that 131.6 gm. of the 150 gm. of glucose ingested were eliminated unburnt.

The nitrogen metabolism was diminished by a little more than 5 gm. on the fourth day and was reduced almost fifty per cent. on the sixth and seventh days. If the increase in the protein metabolism in phlorhizin diabetes were due to dynamogenetic reasons only, the burning of 5.5 gm. of glucose on the fourth day could not have spared the combustion of 31.8 gm. of protein. Nor could the burning of 18.4 gm. of glucose on the sixth and seventh days have spared as much as 81 gm. of protein.

From this experiment it is apparent that in phlorhizin diabetes, extra protein is catabolized in order to maintain the glucose concentration of the blood which, perhaps for some physico-chemical reason, is essential to the processes of life. The introduction of glucose into the system, although very little of it is burnt, spares that amount of protein.

It is also noteworthy in this experiment that the 150 gm. of glucose given within 12 hours were not eliminated completely during the first 24 hours, but were carried over to a great extent to the second 24 hours.

38 (647)

The influence of glutaric acid on phlorhizin diabetes.

By **A. I. RINGER.**

[*From the Department of Physiological Chemistry of the University of Pennsylvania, Philadelphia, Pa.*]

Baer and Blum found that the subcutaneous injection of 10 gm. of glutaric acid had the power of greatly reducing the amount

of sugar and nitrogen in the urine of phlorhizinized dogs. The degree of reduction in the nitrogen elimination in most of their dogs is so marked (less than 1 gm. per 24 hours for dogs weighing 5.4 to 10.0 kilos!) that a repetition of this experiment seemed desirable.

Thanks to the kindness of Prof. Graham Lusk, I received 100 gm. of glutaric acid, which was prepared by Kahlbaum, and which enabled me to carry out the following research:

Dogs were phlorhizinized in the usual manner, and after establishing the D : N ratio, they received, subcutaneously, 10 gm. of glutaric acid dissolved in water and neutralized by means of $NaHCO_3$. The glutaric acid was administered in three equal doses during the course of the day.

Dog No. 5.

Weight.	Total N.	Total Sugar.	D : N.	Remarks.
12.7	18.02	60.96	3.38	
12.2	21.07	70.32	3.33	10 gm. glutaric acid.
11.9	19.75	66.24	3.35	
		Dog No. 7.		
13.87	16.92	63.05	3.72	
	17.86	65.54	3.67	10 gm. glutaric acid.

These results show that the glutaric acid, contrary to the findings of Baer and Blum, has no influence whatsoever either on the sugar or on the nitrogen elimination.

Another experiment was performed on a normal starving animal. It received 10 gm. of glutaric acid without showing any effect on the nitrogen elimination.

Baer and Blum report that a good many compounds containing two carboxyl groups possess the power of reducing the sugar and nitrogen elimination. Experiments are in progress to verify their contention.

39 (648)

Influence of anemia and hyperemia on the growth of sarcoma in the white rat.

By **M. J. SITTENFIELD.**

[*From the Department of Pathology of Columbia University, College of Physicians and Surgeons.*]

From previous experimentation, it becomes evident that certain physiological and pathological factors on the part of the organism, as starvation, pregnancy, the feeding of various salts, racial differences, and so on, exert a decided influence upon the growth of transplanted tumor tissue. Moreschi in his experiments in 1909 concluded that under-feeding and starvation of the animal predisposes to retardation of the transplanted mouse carcinoma. Cluett, Mercier, and others have shown that during pregnancy the progress of the growth of the tumor which may be at a standstill shortly before the ending of pregnancy, is very much lessened, and after labor and during lactation may even recede, *i. e.*, that the growth of one tissue exerts an unfavorable influence upon the artificially transplanted tissue growth of another.

Negré, Borrell's pupil, was able to control the percentage of takes by increasing or diminishing the salt contents of the fluids and tissues of the body. This proves conclusively that the subcutaneously transplanted tumor is dependent upon a great many, as yet unknown, conditions of the host, and also upon its metabolic changes.

The present experiments were undertaken with a view to studying the effect of tumor growth in a locally induced anemic and hyperemic condition in the white rat. The lower extremity of the rat was rendered partially anemic by ligation of the femoral artery at the saphenous opening, and a few hours subsequent, it was observed that the extremity became slightly paler than the opposite one, though there was no evident lack of circulation; and twenty-four, forty-eight, and seventy-two hours after this induced anemia, Ehrlich's rat sarcoma was inoculated subcutaneously into the leg. This experiment was performed on forty animals; of these thirty-three survived four to ten weeks,

and in only six animals, or eighteen per cent., did the tumor grow, and then only to a small size. The same number of controls were inoculated with seventy per cent takes.

To study to what extent anemia was present in these animals, several animals were killed, the blood washed out of both extremities by salt solution, subsequently injected with bismuth, and skiagraphed. These show that there is a marked difference between the vascular supply of the anemic leg, compared to the normal one.

Another set of experiments was performed to study the effect of passive hyperemia of the leg, induced either by rubber ligature, or by a bandage of adhesive plaster strips. A few hours afterwards, the leg became slightly swollen, grayish red, and the arteries still pulsating. This was continued for five to eight days, caution having been taken not to permit edema. Twenty-four hours after the onset of the hyperemia, twenty-five animals were subcutaneously inoculated in the leg with Ehrlich's rat sarcoma, and here the takes were ninety-six per cent.; and the tumor grew more rapidly and to a larger size than in the control animals, in which the takes were sixty per cent.

It is noteworthy that the anemic animals were subsequently inoculated subcutaneously and in only two out of twenty-nine did a growth result. It would seem, therefore, that these animals possessed an acquired immunity, probably from the absorption of substances from the first inoculation.

From these experiments it becomes apparent that partial anemia and passive hyperemia of the leg exert different influences upon the growth of the transplanted tumor, similar to the difference noted by Moreschi in his experiments of over and under feeding of his animals.

Goldman, in his experiments, has shown that the blood supply and the new formation of blood vessels are essential factors in the growth of a cancer cell.

Carl Levin, Bashford, and Gierke, have pointed out that the fibro-plastic and angeoplastic reaction on the part of the host are the deciding factors in the growth of a transplanted tumor cell, and it shall be the aim of subsequent experiments to further study the relation of the angeoplastic reaction on the part of the host in the anemic and hyperemic condition.

40 (649)

Tho elimination and toxicity of caffein in nephrectomized rabbits.

By **W. SALANT** and **J. B. RIEGER.**

[*Presented by permission of the Secretary of Agriculture.*]

In experiments on the elimination of caffein in rabbits and guinea pigs carried out by the writers[1] in this laboratory recently it was found that much larger amounts of caffein were recovered from the gastro-intestinal canal when these animals were fed oats than when carrots were given. Since greater quantities of urine are secreted on a diet of carrots than on one of oats, it seemed probable that the excretory function of the gastro-intestinal canal might be stimulated to greater activity in order to compensate for the diminished diuresis when oats were fed. The elimination of caffein into the gastro-intestinal canal after the removal of both kidneys ought to be greater, therefore, than in normal animals.

In experiments which were performed with caffein which was given subcutaneously to rabbits after double nephrectomy, the following results were obtained: The amounts recovered at the end of about 22 hours from the contents of the stomach and intestines varied between 7.7 per cent. and 11.78 per cent., which is two to three times greater than was found in normal rabbits, much larger amounts being present in the intestines than in the stomach. In one rabbit which died seven hours after caffein was injected subcutaneously, about $10\frac{1}{2}$ per cent. were recovered from the intestines and about half this amount was obtained from the contents of the stomach.

The total amount of caffein eliminated in nephrectomized rabbits in about 22 hours was approximately equal to that eliminated by the gastro-intestinal canal and kidneys combined of normal rabbits during an equal period of time, thus showing that the stomach and intestines acquire much greater power of elimination after the kidney is removed.

Observations were also made on the toxicity of caffein in nephrectomized rabbits. The results obtained showed that the resistance

[1] Bull. 157, Bur. of Chemistry.

is not less than in normal rabbits. In fact, it showed rather a tendency to greater resistance after the kidneys had been removed. Thus 100–150 milligrams of caffein per kilo failed to produce symptoms in nephrectomized rabbits. As was shown by the writers elsewhere,[1] 15 omilligrams per kilo injected subcutaneously into normal rabbits are usually toxic. A dose of 200 milligrams per kilo proved fatal to one rabbit, but two others survived with the manifestation of symptoms. It is interesting to recall in this connection that similar results were obtained several years ago by Meltzer and Salant[2] in experiments with strychnin in nephrectomized rabbits.

41 (650)

A quantitative study of the pupil dilatation caused by adrenalin.

By **DON R. JOSEPH.**

[*From the Department of Physiology and Pharmacology of the Rockefeller Institute for Medical Research.*]

In the normal rabbit, adrenalin given subcutaneously has no effect on the pupil; if given intravenously in fairly large doses there may be a dilatation lasting less than a minute. S. J. Meltzer and C. M. Auer have shown that after removal of a superior cervical sympathetic ganglion in rabbits, the pupil of the corresponding side dilates maximally upon the administration of adrenalin either subcutaneously, intravenously or by instillation. Their experiments were carried out from the qualitative point of view, that is, fairly large doses of adrenalin were used and a wide, long-lasting dilatation of the pupil on the gangliectomized side resulted.

I have recently made a quantitative study of the effects of intravenous injections of adrenalin on the pupil after removal of a superior cervical ganglion in rabbits. The object was to determine the minimal dose that will give a dilatation, and also the amount and duration of the dilatation produced by larger doses. The doses of adrenalin used per kilo animal, expressed in c.c. of the 1/1,000 commercial adrenalin solution, were 1/50, 1/30, 1/20, 1/10 and 2/10 c.c.

[1] Bull. 148, Bur. of Chemistry.

[2] *Jour. Exp. Med.*, 1901, Vol. 5, p. 643.

The results, stated briefly, are as follows: The average pupil-dilatation in six experiments with 1/50 c.c. of adrenalin per kilo animal was 1.62 mm., with a beginning recovery from dilatation in four minutes, and a complete recovery in ten minutes.

In six experiments with 1/30 c.c. there was an average dilatation of 2.25 mm.; recovery began in eight minutes and was complete in twenty-eight minutes.

In thirteen experiments with 1/20 c.c., there was an average dilatation of 3.61 mm.; recovery began in six minutes and was complete in thirty-seven minutes.

In eight experiments with 1/10 c.c., there was an average dilatation of 3.87 mm.; recovery began in ten minutes and was complete in forty-nine minutes.

Finally, in eight experiments the dosage was 2/10 c.c. Here the average dilatation was 4.25 mm.; recovery began in twenty-five minutes, and was practically complete in an average of one hundred and eight minutes.

In other words, 1/50 c.c. of adrenalin per kilo animal was practically the minimum amount that could be relied upon to give a definite dilatation; as the dosage of adrenalin was increased, the dilatation also became greater, remained at its maximum for a longer time, and the return to a normal diameter was slower.

42 (651)

Intermittent and continuous lights of equal intensity as stimuli.

By **G. H. PARKER** and **B. M. PATTEN.**

[*From the Zoölogical Laboratory of the Museum of Comparative Zoölogy at Harvard College.*]

It is generally assumed that white lights of equal intensity give equal stimulation. We have attempted to ascertain whether there is any observable physiological difference between the action of continuous white light and intermittent white light of equal intensity. From a common source of light two beams were conducted over separate paths of equal length to a common observation point. One beam passed through a narrow slit and was thus reduced to a continuous stream of light of low intensity. The

other beam was reduced by being passed through a revolving sector-wheel, thus giving rise to a succession of flashes and dark intervals which fused indistinguishably in the eye, producing the appearance of a continuous flow of light of low intensity. By adjusting the sector aperture and comparing the lights in a photometer, the two lights could be made physiologically equal. On measuring the physical intensities of the two physiologically balanced lights by means of a radiomicrometer, the intermittent light was found to be about 6 per cent. stronger than the continuous light. When the two lights were made equal from the standpoint of their physical intensity and were compared in a photometer, the continuous light appeared much brighter than the intermittent one. From these results we conclude that intermittent white light is a measurably less efficient stimulus than continuous white light of the same intensity, and that in this respect the action of the retina, like that of the photographic plate, affords an exception to the Bunsen-Roscoe law. The reduced efficiency of intermittent light is probably the result of chemical induction dependent upon the frequent interruptions of the light. The sector wheel (episcotister) is therefore an unreliable means for reducing the intensity of light.

43 (652)

Preliminary communication on the cytolytic action of ox-blood serum upon sea-urchin eggs, and its inhibition by proteins.

By **T. BRAILSFORD ROBERTSON.**

[*From the Herzstein Research Laboratory and the Rudolph Spreckels Physiological Laboratory of the University of California.*]

1. It has been shown by Loeb[1] that the eggs of sea urchins (*Strongylocentrotus purpuratus* and *Strongylocentrotus franciscanus*) may be fertilized by the blood-sera of mammalia, provided the eggs be previously sensitized by a brief immersion in a solution of $SrCl_2$ which is approximately isotonic with sea water.

2. I find that if ox-serum be rendered sufficiently potent by dilution (cf. below) the formation of a fertilization-membrane by

[1] J. Loeb, *Arch. f. d. ges. Physiol.*, 118, 36, 1907; 122, 196, 1908; 124, 37, 1908; "Die chemische Entwicklungserregung des Tierischen Eies," Berlin, 1909, p. 185.

the action of the serum is succeeded by cytolysis or may even be accompanied by marked agglutination of the eggs, thus confirming Loeb's view that the formation of a fertilization membrane is essentially a phenomenon of incipient cytolysis.

3. The cytolytic (and fertilizing) action of ox serum (rendered isotonic to sea water) upon sensitized sea-urchin eggs is enhanced by dilution with sea water, a maximum potency being attained at a dilution of about 1/16.

4. I find that the increase in the cytolytic activity of serum which accompanies dilution is attributable to the fact that the proteins in serum in some degree inhibit membrane-formation. The inhibitory effect of the proteins becomes negligible if these are sufficiently diluted. If a protein (*e. g.*, gelatin or ovomucoid) be added to diluted serum its cytolytic activity is greatly diminished or even abolished.

5. The inhibiting action of proteins upon cytolysis is due to the fact that they penetrate the outer membranes of the cells either with difficulty or not at all, so that by their osmotic tension they prevent the taking up of water by the cells. This is well illustrated by the fact that the order of efficiency of different proteins (the mixed proteins of serum, gelatin, "insoluble" serum globulin, casein and ovomucoid) is the reverse order of their ability to pass through the pores of a porcelain filter. The following table shows the concentrations of the various proteins investigated which were observed to permit or inhibit the formation of spherical membranes after treatment of the eggs with 50 c.c. of sea water containing 2½ c.c. of *N*/10 butyric acid for 2½ minutes and then transferring them to 50 c.c. of the protein solution in sea water:

Protein.	Highest Observed Concentration which Permits the Formation of a Spherical Membrane within 1½ Hours. Per Cent.	Lowest Observed Concentration which Prevents the Formation of a Spherical Membrane within 1½ Hours. Per Cent.
The mixed serum proteins	3.7	7.4
Gelatin	1.0	2.0
"Insoluble" serum globulin	0.3	0.6
Casein	0.25	0.5
Ovomucoid	0.125	0.25

6. It will be observed that the power of the mixed proteins of serum to inhibit membrane-formation is very strikingly inferior

to that of the other proteins investigated. On the other hand the CO_2- or "insoluble" globulin of serum, when isolated and dissolved in sea water, is no less potent than other proteins in inhibiting membrane-formation. A 0.3 per cent. solution of the "insoluble" serum globulin very noticeably inhibits membrane-formation, and yet a 3.7 per cent. solution of the mixed proteins of serum, containing 0.33 per cent. of the CO_2-globulin, under the conditions enumerated above only inhibits membrane-formation to a barely perceptible extent. These facts would appear to lend confirmation to the view advanced by Hardy[1] and myself[2] that the various proteins in sera are not present therein in the free condition, but bound together in a molecular complex.

44 (653)

Preliminary note. — The action of various agents upon the secretion of milk.

By **ISAAC OTT, M.D.,** and **JOHN C. SCOTT, M.D.**

In these experiments we used the lactating goat, obtaining the milk by aspiration with a water bottle. We found, as Mackenzie has noted, an increased secretion from venous injections of extracts of the mammary gland. The boiled gland was also active. The pineal body, corpus luteum, and infundibulin were active after a previous dose of atropin. Atropin and antipyrin greatly decreased the secretion. Pilocarpin and digitalin augmented the secretion. Pilocarpin in large doses was active after a preliminary dose of atropin. Albumoses, peptones, and glucose increased the secretion. Sodium, potassium and calcium chloride increased the secretion. Eserine, muscarine, and nicotine did not augment the secretion. 1/1000 of a drop of infundibulin increased the flow of milk, and 1/100 of a drop caused a marked increase. Infundibulin is a 20 per cent. extract of the infundibular part of the pituitary body.

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[1] W. B. Hardy, *Journ. of Physiol.*, 33, 251, 1905 (Appendix).

[2] T. Brailsford Robertson, *Univ. of Calif. Publ. Physiol.*, 4, 25, 1911; "Die physikalische Chemie der Proteine," Dresden, 1912, pp. 126–133.

45 (654)

Preliminary note on the pineal gland and the corpus luteum.

By **ISAAC OTT, M.D.**, and **JOHN C. SCOTT, M.D.**

In a series of experiments we have found the pineal gland to have a marked diuretic action, previously noted by Eyster. At the time of each injection per jugular the volume of the kidney increases considerably, whilst, after a temporary fall, the pressure in the carotid shows some increase. About one half of one per cent. of glucose appears in the urine after the injection of pineal extract and after the use of corpus luteum.

Corpus luteum does not markedly change the pulse rate, but lowers blood-pressure 20–40 millimeters of mercury, and then it rises above normal for a short time. The intestinal peristalsis is markedly increased by corpus luteum.

In the pregnant uterus, corpus luteum increased the contractions.

46 (655)

The spleen and chronic constipation.

By **ISAAC OTT, M.D.**, and **JOHN C. SCOTT, M.D.**

In the *Medical Bulletin*, 1897, one of us (Ott) stated, the spleen of all the animal extracts has the most marked effect on peristalsis. It produces the largest peristaltic waves. When the spleen was removed then peristalsis decreased. If now spleen extract was injected, then peristalsis was restored to a considerable extent above normal.

In 1908, Zuelzer (Dohrn, Marxer and Zuelzer, "Specifische Aufregung der darm Peristaltik, etc.," *Berliner Klinische Wochenschrift*, 1908, No. 48) with others confirmed the preceding results. He prepared an extract of the spleen, called " hormonal," for intravenous and intramuscular injection in man for the cure of chronic constipation. He reports marked success in this condition. This statement has been confirmed by Saar, Henle, Unger and several others.

We have tried the action of the Zuelzer extract of the spleen by the Magnus method. This consists in immersion of an excised segment of the intestine from an etherized animal in Ringer's solution through which oxygen is bubbling. The intestine is attached to a heart-lever and the contractions registered. The spleen extract showed a marked action. In another method a balloon was inserted into the small intestine of an etherized animal and the contractions registered by Albrecht's piston recorder. This method also exhibited an increase of contractions in the intestine. But they are not so marked as when a watery filtered infusion of the spleen was used.

47 (656)

The sequence of the protozoan fauna of hay infusions.

By **LORANDE LOSS WOODRUFF.**

[*Sheffield Biological Laboratory, Yale University.*]

1. In hay infusions, seeded with representative forms of the chief groups of Protozoa, there is a definite sequence of appearance of the dominant types at the surface of the infusion, *i. e.*, Monad, Colpoda, Hypotrichida, Paramœcium, Vorticella and Amœba.

2. The sequence of maximum numbers and of disappearance is identical with that of appearance, except that apparently the position of Amœba advances successively from the last (sixth) place to the fifth place and then to the fourth place.

3. A definite sequence of forms is not apparent at the middle or bottom of the infusions.

4. The middle of the infusions is tenanted chiefly by a free-swimming population brought there by an overcrowding at the top or bottom.

5. All of the protozoan forms considered (except Amœba) are chiefly surface dwellers and it is evident that when they pass their greatest development at the surface this maximum is seldom approached again, and their cycle is practically over.

6. The major rise and fall in numbers at the surface are usually about equally rapid, though the final disappearance of an organism may be long deferred.

7. The appearance of any of the protozoan forms under consideration, excepting Amœba, in appreciable numbers at the bottom is most often coincident with, or immediately subsequent to, its surface maximum, and portends its more or less rapid elimination as an important factor in the life of the infusion.

8. Numerous abnormal individuals and cysts are frequently to be found at the bottom in great abundance immediately after the surface maximum.

9. Emphasis is put upon the strictly biological interrelations (*e. g.*, those involving food and specific excretion products) of the various forms as the most important determining factors in the observed sequence.

48 (657)

The experimental demonstration of the identity of so-called Brill's disease to typhus fever.

By **J. F. ANDERSON** and **J. GOLDBERGER.**

[*From the Hygienic Laboratory.*]

The rhesus monkey is susceptible to infection by inoculation with the blood from a case of "Brill's disease." One attack of the disease in the monkey induces a definite immunity to a subsequent infection with virulent blood of the same strain. Monkeys recovered from an infection with "Brill's disease" have been found to be immune to a subsequent infection with virulent blood from a case of Mexican typhus fever. Monkeys recovered from an infection with Mexican typhus fever have been found to be immune to a subsequent infection with "Brill's disease."

From the above results we conclude that the disease described by Brill is identical with the typhus fever of Mexico, and inasmuch as the New York strain is undoubtedly of European origin, we may also conclude that the typhus of Europe and the tabardillo of Mexico are identical. If this conclusion is correct, typhus fever has been present in New York City for a number of years and, according to verbal reports made to us, has occurred in other large cities of the United States. These results make the clinical recognition and study of typhus fever of increased importance and necessitate the exercise of appropriate prophylactic measures.

It is not intended to exaggerate the menace of this disease to the public health. Nevertheless, although the disease in New York City has apparently been mild and has shown little tendency to spread, it is apparently on the increase there and the possibility should be borne in mind that it may acquire virulence and epidemic prevalence.

49 (658)

The relation of the parathyroid glands to electrical hyperirritability.

By **HERBERT B. WILCOX.**

[*From the Laboratory of Clinical Pathology of the College of Physicians and Surgeons, N. Y. C.*]

The following observations were undertaken for the purpose of obtaining information as to the relation between parathyroid lesion and infantile tetany and of the value of the galvanic reaction as a diagnostic sign of the disease.

In all 25 operations were done on 18 dogs, tests being carried on during August and September, 1910, and from March, 1911, to the present time.

Information was sought on the following points:

1. The limits of response to be expected in peripheral galvanic stimulation of the normal dog.

2. The influence of age upon this galvanic response.

3. The comparability of electrical response in the dog to that of man.

4. The incidence of electrical hyperirritability and tetany following injury to or removal of 1, 2, 3 or all of the parathyroid glands.

5. The time of appearance of these evidences after operation.

6. The length of time by which the electrical evidences precede the physical signs of tetany in their appearance.

7. The constant or varying qualities of the changes of response to electrical stimulation.

8. The frequency of the presence of the electrical evidences of tetany when all other signs are absent.

9. The predisposition to more sudden and severe electrical irritability, when further parathyroid loss is suffered, by animals previously subjected to parathyroid injury.

10. The influence of gestation on the galvanic response of parathyropriva animals.

Technique.—Ether anesthesia.

Three procedures or a combination of the first two were followed.

1. The parathyroid gland or glands were exposed, lifted on the point of a needle and excised or crushed.

2. A thyroparathyroidectomy of one side was done.

3. A complete thyroparathyroidectomy was done on both sides and thyroid extract given to the dog.

For the galvanic tests the peroneal nerve-muscle group was used. The negative electrode was placed on the upper abdomen, the positive over the peroneal nerve as it passes around the head of the fibula. The instrument employed supplied a galvanic current from dry cells and was provided with a rheostat, polarity switch and balanced milliampere-meter measuring from .2 to 10 milliamperes.

Findings.—

1. In normal adult dogs K.C. is usually less than 2 m.a. and may vary between 1 m.a. and 3 m.a. A.C. is usual at from 3 to 5 m.a. It is seldon less than three and often greater than 5 m.a. A.O. is rarely obtained at less than 5 m.a. K.O. is never below 5 m.a.

2. Young puppies are less susceptible to galvanic stimulation than grown dogs. K.C. and A.C. alone are obtained at less than 5 m.a. Frequently K.C. alone is obtained and not often below 3 m.a.

3. Comparison of the findings on normal and parathyropriva dogs with over 300 observations on normal children and children having tetany shows that galvanic response is very similar in the two classes.

4. Of 4 dogs with one parathyroid gland removed, none developed frank[1] tetany. Two showed mild anodal hyperirritability

[1] By frank tetany is meant the development of tremors, muscular or laryngeal spasms, convulsions or paralyses.

during a period of 10 days following operation. One showed moderate anodal hyperirritability during a period of 7 days following operation. One showed no electrical change during 11 days after operation. The removal of one parathyroid resulted in only moderate lowering of the anodal reactions.

Of 8 dogs with 2 parathyroids removed none developed frank tetany. Two showed marked electrical hyperirritability to K.C., A.C., A.O. and K.O. during 25 days following operation. Two showed marked anodal irritability during 13 days and 10 months respectively after operation. Four showed very slight anodal irritability during periods of from 1 to 4 weeks after operation. Electrical irritability of varying degree follows injury to or removal of 2 parathyroid glands.

Of 2 dogs with 3 parathyroids removed neither developed frank tetany. Both gave prompt and marked anodal irritability with occasional low response to K.O. Observations were continued for 36 days after operation.

Of 4 dogs subjected to complete thyroparathyroidectomy at one operation, two gave complete hyperirritability on day following operation and developed tetany two days later. One developed marked anodal irritability with tetany the following day. One, for 46 days, showed neither electrical nor other symptoms of tetany.

Of 7 dogs having part of their parathyroid tissue removed at one time, and the remainder later, 5 developed prompt and complete electrical change and frank tetany. One showed no hyperirritability but died of tetany in six days after operation. One showed complete galvanic reactions but died on the sixth day without other signs of tetany. Prompt and complete hyperirritability, followed in from one to two days by severe tetany, resulted in the removal of all parathyroid glands.

5. Electrical change was noted in from 5 to 48 hours after operation. The promptness and severity of its development depended to a considerable degree upon the amount of parathyroid tissue removed.

6. In all dogs developing frank tetany the electrical diagnosis was established from 1 to 3 days before other symptoms appeared. Lesions which failed to produce tetany were usually accompanied

by electrical change. Marked hyperirritability was present in one case for 11 months without other symptoms of tetany.

7. Sixteen dogs at some time during their periods of observation gave response to all forms of current at less than 5 m.a. Seven dogs with all parathyroids absent were constantly low in all reactions. Five dogs, 2 with all glands removed, 3 with 2 removed, 1 with 3 removed, were constantly low in all but K.O. Four dogs, 3 with 2 glands removed, 1 with 3 removed, gave constantly varying reactions.

In one instance there was constant low K.C., high K.O. and great variation in the anodal tests.

As a rule the electrical change is sufficiently constant to make the test a valuable one. Variations are, however, to be expected.

8. Eight dogs, 2 with 1 gland, 4 with 2 glands, 2 with 3 glands removed showed marked and complete electrical hyperirritability without other symptoms of tetany. Parathyroid injury sufficient to produce hyperirritability does not necessarily result in frank tetany.

9. Eight dogs were subjected to parathyroid injury and later to further loss of glands. In each instance the electrical changes following second operation were more rapid in development and more severe than those noted after the first operation.

10. While under observation 2 parathyropriva dogs littered normally and 1 aborted at about 1 month. None developed tetany during gestation or lactation. There was moderate increased irritability during gestation which became more marked during lactation. No electrical change occurred in the animal which aborted.

SCIENTIFIC PROCEEDINGS.

ABSTRACTS OF THE COMMUNICATIONS.

Forty eighth meeting.

Cornell University Medical College. April 17, 1912. President Ewing in the chair.

50 (659)

The influence of alcoholism on the offspring.

By **CHARLES R. STOCKARD.**

[*Department of Anatomy, Cornell University Medical College, N. Y. City.*]

Two years ago I showed that almost all known gross deformities of the brain could be produced by treating developing fish embryos with alcohol and a number of anæsthetics.

Since that time these experiments have been extended to birds and mammals. The work of Feré with hen's eggs has been repeated and his results confirmed. When these eggs are subjected to the fumes of alcohol the shell is penetrated and the developing embryo is affected. The rate of development is reduced and a large number of monstrosities occur.

Guinea pigs have been put into a state of chronic alcoholism by treating them for six days per week with alcohol fumes to almost the point of intoxication. Forty full-term matings of various combinations have been made with these alcoholic animals. Treated males have been paired with normal females (test of paternal influence on offspring), treated females paired with normal males (maternal influence plus the direct effect on the developing embryo) and finally treated males and females were paired. The outcome of these matings has been most striking.

Twenty-five matings gave no result or the embryos were aborted early and eaten by the mother. Fifteen matings produced in all 25 young, of these two have lived to reach maturity and are apparently normal, four are still young but seem normal. Of the other 19, eight were stillborn or aborted shortly before

term, seven lived for a few days after birth and all died in convulsions, four were *in utero* when the mothers were killed and one of these was deformed.

All of the control matings were successful, all of the young lived and were vigorous.

51 (660)

Growth and maintenance on purely artificial diets.

By **THOMAS B. OSBORNE** and **LAFAYETTE B. MENDEL.**

[*From the Laboratory of the Connecticut Agricultural Experiment Station, and the Sheffield Laboratory of Physiological Chemistry in Yale University, New Haven, Connecticut.*]

[*With the coöperation of the Carnegie Institution of Washington.*]

In earlier reports of the authors' feeding experiments with isolated food substances[1] attention was directed to the failure to induce growth or secure prolonged maintenance of body weight in albino rats with any of the food mixtures tried prior to the introduction of "protein-free milk" as the adjuvant of the dietary which furnished the inorganic nutrients together with some of the carbohydrate (in the form of lactose). In order to determine whether the nutritive success achieved by the use of the protein-free milk was due to the peculiar supply of inorganic salts or some other ingredient, an artificial mixture of salts was prepared to imitate as nearly as possible the proportions of acid and basic radicals in the milk product. This mixture, the preparation of which will be described in detail in a forthcoming paper, contains: Ca 1.97; Mg 0.23; Na 2.03; K 2.66; PO_4 3.33; Cl 4.13; SO_3 0.30; Fe 0.04; citric acid 3.33; lactose 82.0 per cent. This purely artificial product added to purified proteins, starch, sugar and lard has already sufficed to meet the needs of rats for maintenance over very considerable periods of time, and has, thus far, proved as efficient in promoting early growth as the so-called protein-free milk used in our former experiments.

[1] Osborne, T. B., and L. B. Mendel, Carnegie Institution of Washington, Publication 156, Part II, 1911; and *Science*, 1911, XXXIV, p. 722.

52 (661)

Feeding experiments with fat-free food mixtures.

By THOMAS B. OSBORNE and LAFAYETTE B. MENDEL.

[*From the Laboratory of the Connecticut Agricultural Experiment Station, and the Sheffield Laboratory of Physiological Chemistry in Yale University, New Haven, Connecticut.*]

[*With the coöperation of the Carnegie Institution of Washington.*]

The question as to whether fats are, like proteins and carbohydrates, in some measure indispensable components of the diet has never been adequately determined. Stepp[1] has lately maintained that the so-called "lipoids," in distinction from true fats, are necessary for adequate nutrition. His experiments were conducted with mice. Following the methods employed by the writers[2] it has been possible to induce rats to grow at a normal rate with food mixtures containing only purified proteins, carbohydrates and inorganic salts. The problems suggested by the possibilities of this method of investigation are obvious.

53 (662)

The masking of a Mendelian result by the influence of the environment.

By T. H. MORGAN.

[*From the Department of Zoölogy, Columbia University.*]

As reported (Oct., 1911) a mutant of *Drosophila* appeared with a dominant sex-linked character, viz., abnormal abdomen. Typical Mendelian ratios are found in the F_2 offspring if an abundance of food and of moisture is present. As the culture grows older the flies that emerge later gradually change over to the normal type. As a result the Mendelian ratio completely disappears from the surface phenomena. That Mendelian inheritance has actually occurred, but is temporarily masked, is shown by testing the F_2 flies, when the expected number is found (under wet

[1] Stepp, *Zeitschrift für Biologie*, 1911, LVII, p. 135.

[2] Osborne, T. B., and L. B. Mendel, "Feeding Experiments with Isolated Food-Substances," Carnegie Institution of Washington, Publication 156, 1911.

conditions) to transmit the abnormal abdomen. This is best demonstrated by linking the factor for abnormal abdomen *A* with another sex-linked factor, such as the *b* factor in the yellow mutant, or the *c* factor in the white mutant, or to both together as in the two examples given below.

YRA♂ \ *GRA*♀ / *GWN*♀ ╱ *GWN*♂ =	*GRA*♀	95	56	3
	GRN♀	0	4	32
	GWN♀	88	49	34
	GWN♂	98	49	19
	YRA♂	81	47	7
	YRN♂	1	2	25

YRN♀ \ *GRN*♀ / *GWA*♂ ╱ *YRN*♂ =	*GWA*♂	50	21	9
	GWN♂	1	24	24
	GRA♀	76	3	0
	GRN♀	5	49	60
	YRN♀	45	93	61
	YRN♂	49	29	30

The tables show that in the expected classes for abnormal abdomen *A* this character at first appears but later is replaced by the normal character *N*. When the normal flies (of the last sort) were tested they were found to transmit abnormality. The other classes that are genotypically normal remain so in the next generation. Cultures that had produced only abnormal flies for nine generations under wet conditions were allowed to dry out when all the later-hatched flies became normal. These were then placed under moist conditions, and all of their offspring were as abnormal as their ancestors had been.

54 (663)

Sources of error in serological work.

By **WILFRED H. MANWARING.**

[*From the Rockefeller Institute for Medical Research.*]

I have been engaged for some time in attempts to determine the approximate chemical nature of certain bactericidal substances obtained from horse leucocytes. My earlier attempts to isolate and identify these substances were characterized by inconstant and inconsistent results. This led to an examination of the

experimental method, with the discovery of certain sources of error not usually taken into account in serological work.

The first source of error is the possibility of there being marked changes in the chemical composition of serological substances as a result of changes in concentration. The bactericidal substance with which I was working is fairly stable. It can be heated to 60° C. for an hour without loss of bactericidal power and can be stored in the ice chest for weeks with but slight deterioration. The substance can be passed through a series of chemical manipulations, involving such processes as salting-out, dialyzing, evaporating to dryness, and redissolving, and can be recovered quantitatively from the final product of such manipulations, provided the volume of fluid in which it is dissolved is at no time allowed to increase much above the original volume from which the substance was obtained. If the volume is allowed at any stage to materially increase, there is brought about a rapid deterioration of the bactericidal substance at that stage, giving a final product without bactericidal action.

The second source of error is the possibility of there being marked changes in the specific properties of serological substances as a result of variations in the amount of sodium chloride with which they are mixed. The purified bactericidal substance from horse leucocytes, dissolved in distilled water, has about half the bactericidal power of the initial crude product. If dissolved in physiological saline solution, instead of in distilled water, it is without bactericidal power.

55 (664)

The relation of the virulence of the tubercle bacillus to its persistence in the circulation.

By **ALFRED F. HESS.**

[*From the Research Laboratory, Department of Health, New York City.*]

It seems as if the tubercle bacillus offered an exceptional opportunity to study the question presented in the title of this study. As is well known, one type of the bacillus, namely, the human type, is non virulent for the rabbit, whereas the bovine type causes

a general tuberculosis in this animal. Accordingly experiments were instituted in the following manner: four rabbits were injected for each test, two with a human type of bacillus, the other two with the bovine type, in each case 1/100 mg. and 1 mg. of a culture being injected into the ear vein. These four animals were bled one half hour, one hour, two hours, and three hours after injection, 5 cu. cm. being caught in a solution of sodium citrate. There were thus eight specimens taken from the two rabbits inoculated with the human tubercle bacillus, and eight from the two inoculated with the bovine bacillus. These sixteen specimens were injected into as many guinea-pigs, and after six weeks these animals were examined for tuberculosis.

In all, six experiments of this description were successfully carried out, using twenty-four rabbits and 112 pigs. In the tuberculosis test, 44 of the pigs injected with "bovine blood" survived, 26 of these, that is, 69 per cent., were found tuberculous. Of 45 of those injected with the human virus 7 developed tuberculosis, that is, about 18½ per cent. In almost all of these experiments the bovine bacillus was found more frequently in the circulation than the human type of bacillus. This was not the case to any great extent in two of the experiments, where the bovine strain was not of marked virulence.

The conclusion is, therefore, that a certain parallelism exists between the virulence of the tubercle bacillus and its persistence in the circulation of the rabbit. The more virulent bovine organism remains in the circulation more constantly and for a longer period than the less virulent human type. The cause of this difference is being studied at present. Perhaps we can generalize from this fact in the case of other microörganisms.

56 (665)

On indican in the blood of uremic patients.

By **NELLIS B. FOSTER.**

[*From the medical service of the New York Hospital and the Laboratory of Biological Chemistry, Columbia University, at the College of Physicians and Surgeons, New York.*]

A demonstration of the constant presence in uremic blood of an abnormal aromatic body such as indican would be highly signifi-

cant since this would be suggestive of the nature of the abnormal metabolism. Hence a recent report[1] to the effect that indican is present in the blood in uremia invited confirmation, and especially as the amounts present are stated to be sufficient to give the ordinary blue color with chloroform when only 10 c.c. of serum are used for the test. Considering the total volume of blood this would mean an enormous concentration.

The method for detecting indican employed by Obermayer, was, in brief, to separate all of the proteins from the serum by means of alcohol, which after filtration is evaporated on the steam bath. The residue from the alcohol filtrate is taken up in water, freed of salts with lead acetate and the latter in turn removed by sodium phosphate. A water clear filtrate is the final result which is tested by the usual method employed for urine with Obermayer's reagent.

In repeating Obermayer's experiments the method above mentioned was used, also the separation of the proteins was conducted by means of phosphotungstic and hydrochloric acids and in a third series a method was used which is based on that of Rona for the precipitation of colloids in blood, by means of kaolin. The clinical material consisted of ten typical cases of uremia, all of the convulsive type. It was noted that indican could not be detected in fresh serum when 10 c.c. was used for tests; with larger amounts, 25 c.c., a questionable coloration of the chloroform resulted in one instance. If instead of using fresh serum or blood, the material be allowed to stand twenty-four to thirty-six hours many of the uremic bloods then gave a fairly definite reaction. When the kaolin method of separation of proteins is employed, however, no indican could be detected even though the equivalents of as much as 50 c.c. of blood be used for the test.

[1] Obermayer and Popper, *Zeitschr. f. Klin. Med.*, 1911, 72, pp. 333–72.

57 (666)

The inhibitory action of adrenalin in muscle-pancreas mixtures.

By **ALWIN M. PAPPENHEIMER, M.D.**

[*From the Department of Pathology, College of Physicians and Surgeons, New York City.*]

Much evidence has accumulated since Blum's discovery of adrenalin glycosuria in 1901, to indicate an antagonistic action between the adrenal and the pancreas in carbohydrate metabolism. The more intimate nature of this antagonism is still obscure. Bayer in a recent article has suggested three possible modes of interaction:

1. The action of the internal secretion of the pancreas may be directly inhibited by the adrenalin, or

2. The secretion of the pancreatic hormone may be prevented, either directly or through reflex nervous influence, or

3. The adrenalin, through its vaso-constrictory action, may close up the channels of exit through which the internal secretion of the pancreas reaches the circulation.

At the suggestion of Professor MacCallum, the attempt was made to demonstrate this antagonistic action between adrenal and pancreas in vitro, where the problem is simplified by the exclusion of secretory or vaso-motor influences. The technique used was, with slight modifications, that described by Cohnheim in 1906 for the demonstration of the activating effect of pancreas extract upon the glycolytic ferment of muscle. The finely hashed muscle of cats was extracted in iced sodium oxalate solution, the excess of oxalate precipitated with calcium chloride, and glucose added in known amount. Duplicate flasks of muscle extract alone, of muscle extract plus pancreas, and of muscle extract plus pancreas plus adrenal gland extract or adrenalin in varying dilutions up to 1 in 110,000, were incubated overnight at 37° under toluol. Sugar determinations were made with Benedict's solution from samples taken before and after incubation.

It was found that the addition of adrenalin to muscle-pancreas mixtures prepared in this way, gave a higher sugar content than the controls. This effect was obtained definitely in 9 out of 10

experiments. In 6 of the 10 positive experiments, there was an actual increase in the amount of reducing substance during the course of the experiment.

The results may be summarized as follows:

Mixture.	Average Loss of Reducing Substance.
Muscle-extract alone	− 0.096 gm. in 100 c.c.
Muscle-pancreas	− 0.225 gm. in 100 c.c.
Muscle-pancreas-adrenalin	+ 0.01 gm. in 100 c.c.

These experiments then show that the antagonistic action between adrenalin and pancreas, as schematized by Falta, Eppinger and Rudinger, may be demonstrated in vitro independently of any possible nervous influence. King in 1910 showed that a similar retarding influence upon the disappearance of reducing substance in muscle pancreas mixtures was exerted by thyroid extract.

The question as to whether there occurs in this reaction, a true glycolysis, or as Levene and Meyer hold, merely a condensation of the sugar molecule, is left untouched by these experiments. The antagonistic action of adrenalin and pancreas in regard to the disappearance of reducing substance in muscle extracts seems to be clearly demonstrated.

58 (667)

The characteristic course of the rise of blood pressure caused by an intraspinal injection of adrenalin.

By **J. Auer** and **S. J. Meltzer.**

[*From the Department of Physiology and Pharmacology of the Rockefeller Institute.*]

An intravenous injection of adrenalin causes a rapid steep rise of the blood pressure with a gradual fall. An intramuscular injection produces a similar effect. A subcutaneous injection either produces practically no effect or it causes a very slow rise which rarely exceeds fifteen millimeters. In recent years adrenalin was injected into the spinal canal in conjunction with some local anesthetic. The question as to the nature of the effect of these injections upon the blood pressure has to our knowledge never yet been investigated. On the basis of the generally accepted assumption that the absorption from the spinal canal into the circulation

is not very prompt we might expect that the intraspinal injection of adrenalin will have no stronger effect upon the blood pressure than that of a subcutaneous injection. We studied this question experimentally and may say at the outset that our results did not bear out this anticipation. Our experiments were made on six monkeys, using each monkey two or three times. The amount injected was either 1 c.c. or 1.5 c.c. of the commercial adrenalin. Most of the injections were made in the lumbar region; but in a few instances the adrenalin was injected in the thoracic region in the fifth intervertebral space. During the experiments the animals were under fairly profound anesthesia and we are unable to state whether the injection had any other effect besides the change in the blood pressure. But it is important to point out that even doses of 1.5 c.c. of adrenalin had no recognizable after-effects upon the animal.

The action upon the blood pressure was in most cases very characteristic. The pressure would begin to rise slowly but steadily, so that in a few minutes it would reach a maximum varying between 150 and 190 millimeters, and would then commence to go very gradually down. As a rule the entire course of the rise lasted longer than in intravenous injections, in some instances even longer than half an hour. The fall of blood pressure occurred so slowly at times that the original level was not reached during the entire time of observation, a fact which might be of considerable practical importance.

Twenty-one injections were given to these six monkeys at intervals; of these thirteen gave the typical rise described. In six cases the rise was preceded by a moderate fall (9–52 mm.) of short duration (½–4 minutes) and in one case this fall was the only effect of the injection. In the seven instances the injection brought on a rise similar to that of an intravenous injection but of longer duration. It is possible that in these cases part of the injection entered indeed into a vein.

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Demonstration of vomiting movements in an eviscerated animal under the influence of digitalis.

By **C. EGGLESTON** and **R. A. HATCHER.**

[*Laboratory of Pharmacology of Cornell University Medical College.*]

It is commonly accepted that apomorphine produces emesis through its action on the center in the medulla concerned in emesis, but that digitalis produces emesis through its irritant action on the stomach.

We had some evidence that digitalis produced vomiting through its central action and decided to carry out as many experiments as possible of those which had been made in establishing the seat of action of apomorphine, but substituting digitalis, and in the course of the investigation we utilized a method which has not been described hitherto, and which consists in the removal of the gastro-intestinal tract from the esophagus to the anus, after tying the vessels which supply the tract, and injecting digitalis intravenously after an appropriate interval of time, varying from a few minutes to an hour and a half.

We have produced vomiting movements in about fifty per cent. of the experiments so made, and nausea in all but one of the others, and barring those experiments where the depression from the operation seemed to indicate that nausea could not be induced, the percentage of successful experiments is still higher.

Using apomorphine intramuscularly, we have been able to produce vomiting movements in nine out of ten such experiments on the dog.

60 (669)

The variations of pressure in the pulmonary artery.

By **CARL J. WIGGERS.**

[*From the Physiological Laboratory, Cornell University Medical College, New York City.*]

The systolic and diastolic pressures existing in the pulmonary artery of naturally breathing dogs have not been heretofore investigated. By means of a sensitive pulse pressure instrument

capable of standardization against maximal and minimal valves,[1] it has been possible to fill this gap in the physiology of the circulation. The operative technic was so adapted that (1) normal intrathoracic pressure relations during inspiration and expiration were obtained when the records were taken, (2) artificial pressure changes in the intrathoracic cannula and manometer tubes were obviated, (3) clot formation was minimized and recognized when present, (4) only a small portion of the pulmonary circuit was occluded, and (5) the systemic and right auricle pressures corresponded to that habitually found in animals.

The results of 13 such experiments showed that, during quiet normal breathing the systolic and diastolic pressures fell during inspiration and rose during expiration. The systolic pressure averaged 43.3 mm. in expiration and 31.7 mm. in inspiration, the diastolic pressure 20 mm. in inspiration and 11.9 mm. in expiration. In experiments where the heart rate ranged from 180 to 25 per minute, it was found that the diastolic pressure *decreases* as the heart rate is reduced. The same holds true for the systolic pressure between heart rates ranging from from 180 to 100 or 80 (the latter figure varying in different animals). When the heart becomes still slower the systolic pressure again increases.

During temporary apnea vagi, the maximal pressure dropped 40 to 32 per cent., the minimal pressure increased 10–25 per cent. over that occurring during natural breathing, showing that respiratory movements determine to a pronounced extent the extreme pressures in the pulmonary artery.

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The results of ligation of the pulmonary and cutaneous arteries in the frog.

By **T. S. GITHENS.**

[*From the Department of Physiology and Pharmacology of the Rockefeller Institute for Medical Research.*]

The frog possesses, in the lungs and the skin, two organs for the purpose of respiratory exchange, and it has long been estab-

[1] Wiggers, *Journ. Exp. Med.*, XV, 1912, p. 174.

lished that the skin respiration suffices for his needs at low temperature.

I have attempted to produce asphyxia in frogs by ligating the vessels which carry blood to the lungs and to the skin. It may be well to mention here certain anatomical data. The truncus arteriosus rises from the heart of the frog and divides into a right and left branch which each give off three branches, the carotid, the aorta and the pulmocutaneous. The last divides into two, one of which goes to the lungs and the other, the cutaneous, supplies the skin of the entire trunk. A large branch from the carotid makes a free anastomosis with the cutaneous artery.

At temperatures below 20° C. the frog requires very little gas exchange and I found that ligation of the pulmocutaneous and anastomosis was not sufficient to produce asphyxia, although it deprives the frog of the lungs and most of the skin, leaving only the mucous membrane of the mouth and the skin of the legs for respiratory purposes. If in addition the mouth was excluded, by ligation of the carotid arteries, thus leaving only the skin of the legs, the frogs died in 2–3 days. If the lungs and entire skin were excluded by ligating the pulmocutaneous and the iliac arteries death occurred in about 36 hours in spite of the respiratory exchange through the mucous membrane of the mouth. Frogs in which the respiration of the lungs and mouth was absolutely prevented by keeping them under water, but in which the entire skin was available, lived indefinitely at this temperature.

With an increase of temperature to 28° frogs in which the cutaneous respiration was entirely excluded by ligation of the cutaneous and iliac arteries were still able to live indefinitely. If the lungs and skin of the body were excluded by ligation of the pulmocutaneous and anastomosing arteries, death occurred in about 24 hours, and if the skin of the legs was also excluded by ligation of the iliac arteries, in about 12 hours. If the pulmonary and buccal respiration was prevented by keeping frogs under water, no asphyxia was noted during the five hours of the experiment, but frogs in which the cutaneous arteries were tied and which were kept under water died within 3 hours.

At a temperature of 34°, asphyxia could be caused by excluding the cutaneous respiration. Frogs in which the cutaneous and

anastomosing branches were ligated lived only about 8 hours. If the lungs and most of the skin were excluded by tying the pulmocutaneous and anastomosing arteries, the frogs lived about 6 hours and if the mouth also were excluded by sewing this and the nostrils shut and tying the cutaneous arteries, they lived about 4½ hours. Control frogs kept at this temperature, showed no deviation from normal excepting slight over-excitability.

These results show with how little gas exchange frogs can live and also the large factor of safety with which their respiration is normally provided. Auer and Meltzer have recently shown that dogs could live with a supply of oxygen only one tenth of that which they normally consumed.

The results also show the great increase in the requisite gas exchange with rise of temperature and the inability of the skin respiration of the frog to support life at even moderately high temperatures, at which the lungs and mouth alone are still sufficient.

62 (671)

Variations in the response of different arteries to blood serum and plasma.

By **HUGH A. STEWART** and **SAMUEL C. HARVEY.**

Recent work by Brodie, Sollmann, and O'Connor has shown that the blood contains substances acting on the vasomotor apparatus other than suprarenin. Even before the work of these investigators it had been noticed by Stevens and Lee that the use of defibrinated blood for the perfusion of isolated organs was often unsatisfactory because of the gradual diminution in outflow. This was not investigated thoroughly until 1900 by Brodie. He observed that the injection of blood serum into the jugular vein of a cat caused an immediate fall in blood pressure. The cat's own serum was as efficient in this respect as the serum of any other animal. The cat, however, is the only animal which responds in this manner, for Brodie's experiments were negative on the dog and rabbit. The mechanism in this case appears to be a reflex inhibition of the vasomotor center from excitation of the pulmonary branches of the vagus. The importance of Brodie's work lies in the fact that he was the first to show that plasma and

serum are not identical in their physiological activities, for, whereas the effect just described is produced by serum, plasma is entirely inactive. The process of clotting liberates some substance to which this action is to be attributed.

It was probably these experiments of Brodie which guided O'Connor in the analysis of the constrictor effect produced by blood serum on the hind limbs of the frog. He was able to show that the constriction produced by serum is not entirely due to its suprarenin content. Another substance comes into play which causes constriction and which is present only in the serum.

We have investigated the vasomotor effect of plasma and serum in different vessels of the body, especially in regard to their mode of action.

The methods we employed were the perfusion of the organ with Ringer's solution in a moist atmosphere at the temperature of the body and under a constant pressure. The outflow from the vein was recorded with a signal magnet writing on a smoked paper. Two c.c. of serum or hirudin plasma were injected close to the canula inserted in the artery.

Effect of serum.—The typical effect when injected into the vessels of the limb is to produce an immediate diminution in the rate of outflow. This usually lasts for from five to ten seconds, when it gradually begins to return to normal. The original rate of flow is regained in about five minutes.

A similar result is obtained when the heart is perfused.

Entirely different is the effect which is produced when 2 c.c. of serum are injected into the vessels of the perfused kidney. Instead of a vasoconstriction there is produced a pronounced vasodilatation.

We have first to determine whether the dilatory effect on the kidney and the constrictor effect on the limb vessels are due to the same substance. It can readily be shown that two substances are operating. If the serum is boiled and filtered through a Berkfeldt filter and injected we now get no dilatation of the kidney vessels, but instead a constriction.

As before, the effect of boiled serum is to produce a constriction of the limb vessels.

Precipitation of the protein constituents of serum by alcohol

and the injection of the dried filtrate dissolved in Ringer's solution retains the constrictor substance, but it contains no vasodilator for the kidney vessels.

We are, therefore, led to the conclusion that serum contains a constrictor substance which acts on the limb vessels, the kidney vessels and the coronaries. In addition there is also present a dilator substance acting specifically on the renal vessels. The dilator substance is a proteid, the constrictor substance is not. The perfused kidney after the injection of serum is subject to the action of both a dilator and a constrictor substance. The dilator is the more powerful and masks the action of the constrictor. The constrictor substance becomes manifest after removal of the dilator by boiling or by precipitation by alcohol.

Is the constrictor substance described above the suprarenin quotient of serum? Suprarenin, as is well known, causes constriction by stimulation of the sympathetic nerve fibers. If we add apocodeine hydrochloride to the perfusion fluid we can paralyze the sympathetics and in this way render the preparation insensitive to the strongest solutions of suprarenin. If, however, we inject serum there is still produced almost as great a diminution in the rate of outflow as before. We have thus evidenced that the constrictor substance is not suprarenin, and further, that it is a body which acts directly on the muscle coats.

In regard to the dilator substance acting on the kidney we have found that it still causes an increased outflow after the injection of serum when the perfusion fluid contains apocodeine.

Effect of plasma.—Plasma differs markedly in its action. If hirudin plasma is injected into the vessels of the limb it produces a much less constriction than does the corresponding amount of serum. This result is dependent only upon the amount of suprarenin it contains, for when the sympathetics are paralyzed the injection of serum produces no change in the rate of outflow.

On the kidney vessels, however, plasma produces an even greater dilatation than does serum.

The conclusions which we have been able to draw from these experiments are as follows.

1. That in both plasma and serum there is present a substance which causes dilatation of the renal vessels. This substance is a proteid.

2. That the process of clotting liberates a vasoconstrictor substance which acts on the coronaries, the renal vessels and the limb vessels. It acts directly on the muscle coat and it is not a proteid body.

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General physiological properties of diaphragm muscle.

By **FREDERIC S. LEE** and **A. E. GUENTHER.**

[*From the Department of Physiology, Columbia University.*]

Strips of the diaphragm of the cat, both curarized and non-curarized, have been excised after death and experimented with in moist chambers at room temperature. Their great resistance is demonstrated by the facts that they remain irritable two to three times longer than, and do several times the amount of work done by, a leg muscle, such as the extensor longus digitorum. Moreover, the diaphragm is not paralyzed by curare until long after the leg muscles have ceased to act. A most striking phenomenon is the tendency of the diaphragm strips to yield rhythmic twitches. This is much more pronounced than with the control leg muscles. It may be made manifest by the action of solutions of certain electrolytes, where the twitches are irregular in extent and duration; and by weak faradic currents, which insure more regular responses. With 40 to 100 faradic stimuli in the second, the twitches occur at a rate of from 2 to 4. They are much more marked in non-curarized muscle. When irregularities due to the stimulated current are excluded, the following factors may possibly interact in the production of the rhythmic responses: (1) There may be present the Wedenski effect; (2) the weak stimulus may affect from time to time different groups of fibers within the muscle, the irritability of the groups varying; (3) polarizing factors may be present. The relations of these and other possible factors are not yet established. A strip of diaphragm muscle as a whole has a decided power of rhythmical response, but it is not yet certain whether this power is possessed by the individual muscle fibers.

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The stimulation of nerve-endings in muscle and the theory of receptive substances.

By MARY WHITALL WORTHINGTON.[1]

[*Physiological Laboratory of the Johns Hopkins University.*]

Langley has shown that the stimulating action of nicotine upon muscle is prevented by curare and that this antagonism is exhibited also in muscles in which the nerve fibers have been removed by degeneration. This and other facts have led him to formulate the hypothesis of special receptive substances present in the muscle upon which these drugs and other chemical stimuli exert their action, and furthermore he ventures the generalization, in contradiction of the usual belief, that "in no case do chemical substances have a special action on nerve-endings."

The series of experiments here reported were made at the suggestion of Dr. J. W. Warren to test the above hypothesis. The gastrocnemius muscle of the frog was used and its reactions were studied after immersion in solutions of sodium sulphocyanide and potassium sulphocyanide. The following results were obtained.

1. *Action of potassium sulphocyanide.*—This salt was used in hypertonic solutions (2 per cent. KCNS made up in a Ringer's mixture) and in isotonic solutions (1 per cent. KCNS in water). In both cases solutions of this salt caused an immediate rapid primary contraction followed by a prolonged contracture. This effect was not removed by the action of curare nor by the degeneration of the motor nerve fibers. It was obtained also by the action of other potassium salts, *e. g.*, potassium chloride—and must be considered as an instance of the "potassium contraction" described by other authors. The potassium ions may act upon the supposed receptive substance, but evidently their effect does not depend upon the presence of the nerve terminals.

2. *Action of sodium sulphocyanide.*—This salt was used also in hypertonic and isotonic solutions as in the case of the potassium sulphocyanide. In hypertonic (2 per cent.) solutions it caused a large primary contraction followed by a condition of contracture and by frequent isolated or grouped twitches of a coördinated or

[1] Deceased. Presented by W. H. Howell.

fibrillar character. In isotonic solutions the primary contraction was lacking, but fibrillar twitches occurred, although less frequently than in the hypertonic solutions. Hypertonic solutions (2 per cent.) of sodium chloride failed to give anything more than a small increase in tone. The marked primary contraction as well as the isolated twitches observed in the hypertonic solutions of sodium sulphocyanide were entirely lacking in the muscles of curarized frogs or in frogs in which nerve degeneration had been effected by cutting out a portion of the sciatic nerve. In the latter experiments observations were made upon frogs at varying intervals from 3 to 51 days after section of the nerve. It is noteworthy that the falling out of the contraction caused by the sodium sulphocyanide was observed as early as seven days after section and at a time when a response could still be obtained from the muscle by electrical stimulation of the peripheral end of the nerve. So, also, in the case of curare it was found that the sodium sulphocyanide contraction might be abolished even when the curarization of the animal was incomplete. Two per cent. solutions of sodium sulphocyanide have no stimulating action upon the sciatic nerve when immersed in the solution.

Conclusion.—The stimulating action of sodium sulphocyanide is not simply a sodium effect. The fact that its action is abolished by curare may be explained in accordance with Langley's hypothesis of receptive substances, but the fact that its action is readily removed by de-nervating the muscle is in contradiction to the generalization made by Langley in regard to the improbability of a special action of chemical substances upon the nerve endings in muscle.

Since hypertonic solutions of the sodium sulphocyanide do not stimulate the motor fibers in the sciatic nerve, their action in stimulating the muscle can be explained only on the assumption that they stimulate the nerve endings, if one bears in mind that this stimulating action falls out after the nerve fibers in the muscle begin to degenerate, as well as after administration of curare. It is noteworthy that the stimulating effect of this sodium sulphocyanide upon the nerve-terminals disappears in the incipient stages of the degeneration following section of the motor nerves.

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On the isolation of oöcytase, the fertilizing and cytolyzing substance in mammalian blood-sera. (Preliminary report.)

By **T. BRAILSFORD ROBERTSON.**

[*From the Rudolph Spreckels Physiological Laboratory of the University of California.*]

It has been observed by Loeb that the eggs of sea-urchins (*Strongylocentrotus purpuratus*) may be induced to form fertilization-membranes by immersing them in mammalian blood-sera which have been rendered isotonic with sea-water by the addition of NaCl. Occasionally, if the serum be especially potent, simple immersion of the eggs in the serum suffices to bring about this result, but as a rule previous treatment of the eggs with a sensitizing agent ($SrCl_2$ or $CaCl_2$) is required. Prolonged action of the serum upon sensitized eggs results in the cytolysis of the eggs. Very potent sera usually cause agglutination of the eggs as well, especially of sensitized eggs.

I have isolated from ox-serum a fraction which is extremely potent in fertilizing, cytolyzing, and agglutinating sea-urchin eggs.

To 860 c.c. of fresh, whipped, and centrifugalized ox-serum, which had been rendered isotonic with sea-water by the addition of $2\frac{1}{2}M$ NaCl solution, I added 400 c.c. of 7 per cent. $BaCl_2$. A thick cloud was produced. This mixture after standing for an hour in a warm place until its temperature rose to 37° C. was centrifugalized. The entire precipitate, consisting of $BaCO_3$, $BaSO_4$, and the barium compound of the fertilizing agent and, possibly, of other substances, settled in the form of a cake at the bottom of the centrifuge-tubes. This precipitate was thoroughly drained and then suspended several times in 2 per cent. $BaCl_2$ and re-centrifuged in order to free it from adherent serum. It was then stirred up for an hour in 100 c.c. of $N/10$ HCl and the insoluble residue (probably barium sulphate) was centrifuged out. To the clear fluid thus obtained were added 10 c.c. of 10 per cent. Na_2SO_4 in order to free it from barium. This mixture was allowed to stand for some hours at 50° and then centrifuged. The clear

yellowish fluid thus obtained yielded no precipitate or opalescence upon the further addition of Na_2SO_4 and was therefore free from barium. To this fluid were added 4 volumes of acetone. A light flocculent precipitate was formed at once which settled readily. This was collected upon a hardened filter, washed in alcohol and ether, and dried for $3\frac{1}{2}$ days over H_2SO_4 at 36° C.

The substance thus obtained does not dissolve in sea-water. It dissolves readily in $N/10$ HCl and remains in solution upon neutralization. To this solution sufficient $2\frac{1}{2}M$ NaCl was added to render it isotonic with sea-water and the solution (0.5 per cent.) thus obtained was diluted to 1, $\frac{1}{2}$, . . . , etc., by the addition of sea water. On adding sea water, the mixture becomes very opalescent but the substance is not precipitated.

Eggs of *Strongylocentrotus purpuratus* which have not undergone previous sensitization are fertilized and agglutinated by solutions of this substance in dilutions of from 1 part in 200 to 1 part in 800. Eggs which have been sensitized by immersion for 4 minutes in $M/2$ $CaCl_2$ are fertilized and agglutinated by dilutions of from 1 part in 200 to 1 part in 1,600. Eggs which have been sensitized by immersion for 4 minutes in $M/2$ $SrCl_2$ are agglutinated by dilutions of from 1 part in 200 to 1 part in 25,000. The sensitizing action of $SrCl_2$ and $CaCl_2$ is clearly seen to reside in their power to precipitate the fertilizing agent upon the egg.

Since this substance is thermostable, withstanding 18 hours' exposure to a temperature of 50° without destruction, and there is some reason for suspecting that it is not present as such in circulating blood but is discharged from white corpuscles in shed blood, it would appear to present many analogies to the cytases or cell-liquefying substances found by Metchnikoff in white corpuscles. Accordingly, I propose that it be called "Oöcytase."

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The food factor in hibernation. (Preliminary communication.)

By **SUTHERLAND SIMPSON.**

[*From the Physiological Laboratory, Medical College, Cornell University, Ithaca, N. Y.*]

In those animals that hibernate the condition is generally believed to be brought about mainly by a low external temperature; when the winter cold sets in the animal retires to its burrow or nest and remains dormant until spring. Some, on the other hand, hold that a diminished food supply is the chief, or at any rate, an important cause of hibernation, and my experience with a colony of woodchucks (*Marmotta monax*) during the past winter would appear to support the latter view.

About the middle of September, 1911, eighteen woodchucks, which had been caught in box traps in the neighborhood of Ithaca, and were uninjured, were placed in eight artificial burrows about five feet below the surface of the ground, the object being to study, amongst other things, changes in the nervous system during hibernation. The burrows, which were packed with dry straw, opened into a central court into which food (clover, corn, apples, carrots, etc.) was placed every second day, and it was expected that when the animals began to hibernate the food would cease to be consumed.

In this locality I was told that woodchucks are rarely seen in the open fields later than the first or second week of October, and as the food still continued to disappear after that time, the burrows were opened up and the animals caught and examined to find out their condition, on the following dates.—Oct. 13, Nov. 11 and 27, Dec. 18 and 26. They were found to be quite active on all these occasions, with rectal temperatures somewhere in the neighborhood of 100° F.

The weather up till the end of December had been unusually mild for this climate, and this might possibly have had some influence in maintaining the wakeful condition, but from the beginning of January till the end of March the winter was excessively cold, the air temperature being often below zero fahrenheit,

and on two occasions 16° below. Notwithstanding this low temperature the food was still eaten by the woodchucks.

No food was supplied from Jan. 20 till Feb. 14 when the animals were again examined in the burrows. On that day their tracks in the snow were abundant. Six were found to be in a semitorpid condition, but they did not show the deep narcosis of true hibernation; the others were very active and combative, one having a rectal temperature of 99° F. Although no food had been placed in the inclosure for three weeks, it is still possible that they had some stored in their burrows, or that they ate the straw.

The six which showed some degree of torpor were removed to the laboratory, kept overnight outside the building in a large box amongst straw, and the next day, under ether anesthesia, a small lesion was made in the spinal cord of each. On the day following they were completely awake and active, and remained so until killed at various intervals after the operation. Food was supplied to these as well as to those left behind in the burrows from Feb. 14 onwards; they were inspected at short intervals from that date till the end of March and never showed any tendency to hibernate. The rectal temperature ranged from about 95° F. to 101° F.

These animals were not artificially protected from the weather in any way; the only circumstance in which their condition differed from that of their fellows in the open fields was that food was furnished them. The behavior of this colony would seem to point to the fact, therefore, that the absence of food supply is an important factor in determining the onset of hibernation.

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A rapid method of producing a hemolytic serum.

By **FREDERICK P. GAY** and **J. G. FITZGERALD.**

[*From the Department of Pathology and Bacteriology, University of California.*]

Fornet and Müller[1] were first to suggest the intensive method of immunizing animals by giving large doses of serum for pro-

[1] Fornet and Müller, *Zeitschrift für biologische Technik und Methodik*, Strassburg, 1908, vol. 1, p. 201.

ducing precipitins intra-peritoneally on successive days. Such a method apparently gave rise to a potent precipitating serum and they claim equally successful results in producing hemolysins. These results as regards hemolysins were not confirmed in a later communication by Bonhoff and Tsuzuki.[1]

We have found that one can obtain uniformly a sufficiently potent hemolytic serum for fixation tests by immunizing rabbits with washed sheep blood in a dose of one to two cubic centimeters administered intravenously on three successive days. The hemolytic titer of such a serum *four days after the third injection* is usually 1 to 2,000. In other words, it is possible to produce a thoroughly reliable hemolytic serum in one week by this method.

Further details of this intensive method of immunizing to produce hemolytic sera and also its use in producing precipitins, bacteriolysins, agglutinins, and antitoxins will form the basis of a more extensive communication to be published in the Pathological series of the University of California Publications.

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Note on the effect of the internal secretions upon the secretion of epinephrin.

By **ISAAC OTT** and **JOHN C. SCOTT.**

One[2] of us was the first to show that the adrenal secretion relaxed and inhibited the rhythmic contractions of the intestine. Hoskins has shown that a dilution of 1–400 millions of epinephrin inhibits the rhythmic contractions. It thus becomes the most sensitive test for the presence of epinephrin. We tested its presence by the Magnus method. We injected a few grains of the filtered solution of the different glands into the jugular of the narcotized cat, and drew off, as Cannon has done, some blood from the vena cava above the openings of the adrenal veins, and defibrinated it. Normal blood defibrinated was then applied to a segment of the intestine of a narcotized rabbit and its rhythmic movements recorded. Then the blood, after the injection of the glandular filtrate, was applied to the same intestine and its move-

[1] Bonhoff and Tsuzuki, *Zeitschrift für Immunitatsforschung.*, IV, page 180.

[2] *Medical Bulletin*, 1897, p. 376.

ments registered. It was found that the injection of iodothyrin, parathyroid, infundibulin, thymus and pineal caused a distinct fall in tonus and inhibition of rhythmic contractions, showing the presence of epinephrin beyond normal in the blood. Normal blood of the cat never produces this effect, but stimulates tonus and rhythmic contraction. We have been careful to exclude albumen in these glandular tests, as the foreign albumen in the antithyroid serum of Möbius and in the diphtheritic antitoxic serum in 2 drop doses increases the amount of epinephrin in the blood. As cholin also produces an epinephrin reaction we can only be certain that iodothyrin and infundibulin stimulate the adrenals.

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Standardization of the Wassermann reaction. Attempts to prepare a standard antigen and antibody.

By **J. G. FITZGERALD** and **J. B. LEATHES.**

[*From the Department of Pathological Chemistry, Univ. of Toronto, and Department of Pathology and Bacteriology, Univ. of California.*]

For some time past we have endeavored to determine more exactly the nature of the Wassermann reaction. This led us first to an investigation of the antigen. In this connection Noguchi, and we ourselves, have shown the importance of the substances contained in the acetone precipitate. Incidentally, we have found that an antigen containing these substances (lipoids) is available for use after a period of two years. The exact steps in the production of this antigen differ in certain details from methods heretofore published. The method will appear, shortly, elsewhere. The next step in the work was an effort to produce an antibody to this relatively stable antigen. Three attempts have been made and all were unsuccessful. No evidence of antibody formation could be shown by means of the reaction of fixation or the precipitin reaction. *These lipoid substances were found not to act as antigens.*

Had the production of an antibody been possible, the standardization of the Wassermann reaction could have been accomplished. This was the ultimate object of the work. No method

has been devised that permits of the "lipotropic" content of syphilitic sera being determined by quantitative methods by comparing them with such a standard serum as we have tried unsuccessfully to produce. Noguchi has shown that one can determine the absolute amount of a given syphilitic serum that will give complete fixation with a fixed amount of antigen, under certain conditions, at any given time.

In addition, for purposes of comparison at different times it would be necessary to have a standard antigen and a standard antibody (a "synthetic syphilitic" serum) both of relatively constant potency. These things are at present impossible. Quantitative standardization of the Wassermann reaction is, therefore, not feasible in the present state of our knowledge.

SCIENTIFIC PROCEEDINGS.

Abstracts of the Communications.

Forty-ninth meeting.

University and Bellevue Hospital Medical College. May 15, 1912.
President Ewing in the chair.

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The effect of chemicals on the division rate of protozoa.

By **Gary N. Calkins.**

[*From the Department of Zoölogy, Columbia University.*]

These experiments have been carried out during the past year for the purpose of finding out whether the products of nucleoproteid breakdown have any effect upon the division rate of free-living cells, the ultimate aim being to get some light on the controlling factors of cell division.

Two ciliated protozoa were used. One, *Actinobolus radians* Stein, lives exclusively on a diet of *Halteria grandinella*, another ciliate. The other, *Blepharisma undulans* Stein, lives on bacteria. Four control lines of each have been watched, fed, and the number of divisions recorded daily, and curves based upon the averaged division rates for five day periods, give the fluctuations in vitality of the organisms as measured by the division rates. Periods of depression, and of decreasing and increasing vitality have been clearly marked. Individuals for experimentation were in all cases sister cells of the control lines of the same dates.

The chemicals used included various amino acids, nucleins, and their derivatives, for many of which I am indebted to Dr. Levene and Dr. Walter Jones. In each experiment four strengths of chemical were used after empirical determination of the lethal dose. The number of divisions of all four lines were averaged

for five-day periods for comparison with the controls of the same periods, and all experiments were continued for at least ten days.

The results show that amino acids and their derivatives have but slight effect on the division rate at any period of vitality. The purins and their derivatives have but a slight effect on the division rate when vitality is very low or very high, but a marked effect of increasing the rate when vitality is decreasing (allantoin) or increasing (hypoxanthin, xanthin).

71 (680)

The first onflow and diastolic waves in the venous pulse.

By **E. M. EWING.**

[*From the Department of Physiology of the University and Bellevue Hospital Medical College.*]

Simultaneous records of the contractions of the auricle and ventricle, intraventricular pressure, arterial pulse, and the pulse of the superior vena cava were made.

Previous investigators have not agreed as to the time relations of the 3d positive (Mackenzie's "v") wave, some placing its appearance during ventricular systole, and others believing that it occurs in diastole. This confusion has arisen from the fact that Mackenzie's "v" wave in reality consists of two positive waves which are separate both in time and origin. (Bard described two such waves, but was not definite concerning the time relations.)

The first of these waves has been called the "onflow" wave, and the second, the "diastolic" wave. They are preceded, of course, by the auricular and systolic ("c") waves.

In a series of some fifty dogs, the onflow wave has always appeared just at the end of auricular relaxation, and therefore, during the first half of ventricular systole. The wave is terminated at the very beginning of ventricular diastole. The origin of the wave cannot be ascribed to the passive auricle, nor to the ventricle, the base of which is still moving downward, and which would tend, therefore, to produce a negative, rather than a positive wave. The wave must simply represent the increased pressure

resulting from the onflowing blood, which can now no longer enter the completely relaxed auricle, and must necessarily "back up" into the great veins. The instant ventricular relaxation commences, the base of the ventricle pushes up against the column of blood in the auricle and veins, and thus produces a still greater pressure—*i. e.*, the diastolic rise. In all of the experiments this diastolic rise has occurred synchronous with the beginning of ventricular relaxation, *before* the closure of the semilunar valves. The auricle, of course, is still passive.

The diastolic rise continues until the ventricle has relaxed sufficiently to allow the a-v valves to open, when the increased pressure in the auricle is terminated by the rapid outrush of blood. The resulting diastolic fall then continues until the end of ventricular relaxation, or, in other words, until the ventricle ceases to enlarge and receive the onflowing blood. The blood which cannot enter the ventricle, "backs up" into the auricle and veins, and thus produces the 2d onflow wave (Hirschfelder's "h"), in a manner similar to that in which the 1st onflow wave was originated.

The reason for the non-appearance of the 1st onflow wave in many jugular pulse tracings is that it is partially or completely fused with the diastolic wave. (The point of separation is indicated by a notch in many of Mackenzie's records.) The "a," "s," and "d" waves are essentially impact waves, and therefore travel faster than the onflow wave, which is dependent, for its rate of propagation, upon the rapidity of the onflow of blood from the periphery. The onflow wave appears just after the "s" wave in the lower part of the sup. vena cava, but by the time the effect of the "backing up" of blood has been felt in the veins of the neck, the onflow wave has been overtaken and obscured by the "d" wave.

The above conclusion is supported by figures from several experiments, showing that the onflow wave appeared in the jugular vein .05 second later than in the vena cava. The relative positions of the "a," "s," and "d" waves do not vary in the different regions.

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On the action of the infundibular portion of the hypophysis upon vasodilators.

By **J. AUER** and **S. J. MELTZER.**

[*From the Department of Physiology and Pharmacology of the Rockefeller Institute.*]

By the investigations of Oliver and Schäfer, Howell and others, it has been established that by intravenous injections of an extract of the infundibular portion of the hypophysis a rise of blood pressure is produced, which, however is usually not as strong as the one produced by an injection of adrenalin. It is now generally assumed that, like adrenalin, the rise is produced by a stimulation of the constricting mechanism. On the basis of the hypothesis, that the hypophysis may raise the blood pressure not by a stimulation of the constricting, but by a *depression of the vasodilating mechanism*, we studied the effect of stimulation of the depressor nerve in rabbits soon after an intravenous injection of an extract of the hypophysis. We used for this purpose the pituitrin of Parke, Davis & Co., which is made up from the infundibular portion of the hypophysis. In some of the experiments the pituitrin was heated for the purpose of driving off the chloretone. In some of the experiments both vagi were cut to eliminate the inhibitory action through these nerves.

We may state briefly that in every experiment the irritability of the depressor nerve was either abolished or considerably reduced for a few minutes after the injection of the pituitrin. This was definite even before the rise which follows the injection began to develop and also during an insignificant rise. The larger the injected dose of pituitrin, the longer did the reduction of the irritability of the depressor nerve last. In 12 or 16 minutes, however, the irritability, as a rule, returned to normal. On repeating the injections, the reducing effect upon the irritability of the depressor became less and less pronounced.

We may recall here the fact, observed by Oliver and Schäfer and others, that during the rise of blood pressure from adrenalin, stimulation of the depressor nerve is ineffective. But this applies

only to the strong rise which sets in, as is known, immediately after injection of that substance. With the onset of the descent of pressure the depressor action of the depressor nerve becomes more and more effective. Here the inefficiency of the stimulation of the depressor may be due merely to the inability of the depressor nerve to overcome the high pressure. It is different, however, with the action of the hypophysis. Here the rise of blood pressure develops as a rule, gradually and sometimes it is even preceded by a fall. Furthermore in some instances the rise is comparatively insignificant. Nevertheless in all these conditions there is definite evidence of a striking reduction of the irritability of the depressor. We are therefore, for the present, inclined to look upon the discovered reduction of the irritability of the depressor nerve as a confirmation of our hypothesis that the infundibular portion of the hypophysis reduces the irritability of vasodilators. We may mention that studies upon the vasomotor effects of stimulations of the central end of the vagus nerve in dogs, which we shall not discuss here in detail, seem to furnish a further confirmation of this view. As to the locality where the hypophysis develops its action, whether upon the central or peripheral mechanisms or upon both, we shall not discuss at present.

73 (682)

On the reduction of toxicity of strychnin by the simultaneous administration of large quantities of fluid.

By **I. S. Kleiner** and **S. J. Meltzer.**

[*From the Department of Physiology and Pharmacology of the Rockefeller Institute for Medical Research.*]

In the course of some experiments on adrenalin glycosuria in which the simultaneous injection of blood, serum or lymph with adrenalin was studied, it became manifest that the significance of the factor of dilution had to be previously established. It was thought that the study of the effect of dilution upon strychnin poisoning would throw some light upon this. The definite and instructive results which were obtained will be stated here very briefly.

Controls.—Six rabbits received subcutaneously 0.45 mg. strychnin per kilogram body weight. All six had convulsions, with fatal termination in three. Nine animals received 0.5 mg. per kilo; all had convulsions, terminating fatally in four. In 15 controls, then, a dose of 0.5 mg. strychnin or less per kilo body weight brought on convulsions in every one and a fatal termination in 7; in other words 0.5 mg. strychnin proved to be toxic in 100 per cent. and fatal in 47 per cent.

Strychnin diluted in 100 c.c. of normal saline.—Ten rabbits received subcutaneously doses of strychnin varying between 0.7 and 0.84 mg. per kilo body weight diluted in 100 c.c. of 0.9 per cent. sodium chlorid. Of these only two had convulsions, one of which died. In other words, strychnin in great dilution is considerably less toxic; doses which exceed the minimal fatal dose proved to be toxic only in 20 per cent. and fatal in 10 per cent.

Strychnin and saline injected in separate places.—In a series of eleven rabbits each received subcutaneously 0.5 mg. strychnin per kilo body weight at one point shortly after having been injected with 100 c.c. of 0.9 per cent. sodium chlorid at another place. Of these only three had convulsions and only one died. In other words, when 100 c.c. of saline was injected in another part of the body, a dose of 0.5 mg. per kilo of body weight, which in controls proved toxic in 100 per cent. and fatal in 47 per cent., was toxic only in 27 per cent. and fatal in 9 per cent.

Strychnin subcutaneously and water given by mouth.—Eight rabbits received 0.5 mg. strychnin per kilo body weight subcutaneously shortly after 100 to 150 c.c. of water had been given by mouth. Of these animals only two had convulsions and none died, that is, in these cases 0.5 mg. strychnin proved to be toxic only in 25 per cent. and caused no fatalities. Twelve animals were given strychnin subcutaneously, in doses varying between 0.52 and 0.56 mg. per kilo body weight, receiving at the same time 100 c.c. of water by mouth. Of these animals four had convulsions and none died. In other words even doses which definitely exceed the sure toxic dose of 0.5 mg. per kilo, proved toxic only in 33 per cent. with no fatalities at all, when at the same time, a comparatively large quantity of water was given by mouth.

These experiments seem to demonstrate conclusively that the

toxicity of strychnin is definitely reduced not only when it is administered in great dilution, but also when saline or water is administered nearly simultaneously in other parts of the body, thus, perhaps, diluting the poison within the circulation. These results are of general theoretical interest and have obviously also a practical bearing, neither of which we shall discuss here.

74 (683)

The influence of the infundibular portion of the hypophysis upon the pupil.

By **S. J. MELTZER.**

[*From the Department of Physiology and Pharmacology of the Rockefeller Institute.*]

The action of the extract of the hypophysis is similar to that of adrenal extract in the first place by the influence which both exert upon blood pressure. There seems to be also some similarity in their action upon the uterus and intestines. With regard to the action of hypophysis upon the frog's pupil which, as is well known, becomes definitely dilated by adrenalin, opinions differ. While Cramer and others report a dilatation, Kepinow and Gottlieb recently stated that in their hands the extract of the hypophysis caused a constriction of the frog's pupil. In my own observations upon the enucleated bulbi from *Rana pipiens*, heated and unheated *pituitrin* (Parke Davis & Co., prepared from the infundibular portion of the hypophysis) *in most instances caused a dilatation of the pupil.* The extent of the dilatation varied greatly in various eyes and was never as striking as is observed under the influence of adrenalin. In a smaller number no dilatation of the pupils took place; *but in no instance have I observed a miosis following the bathing of the bulbi in pituitrin.*

A very striking test is the action of adrenalin on the pupil of mammals (rabbit or cats) on the side on which the superior cervical ganglion had been removed 24 hours or longer. If a sufficient dose was used, that pupil showed a maximal dilatation which may last for hours, while the pupil on the normal side remains unchanged. I have studied this test with pituitrin. In six rabbits

in whom one superior cervical ganglion had been removed, either on the right or on the left side, I have injected at various times various doses of pituitrin—1 c.c., 2 c.c. and 3 c.c.—through the marginal ear vein. *At no time did a dilatation of the pupil on the operated side follow these injections, neither soon nor late. Both pupils, however, and especially that of the eye on the operated side, showed a constriction of short duration immediately after the injection.*

Here we meet, then, with a definite difference between the action of adrenalin and pituitrin which, in some cases, might assist in the identification of the nature of the blood-raising principle found to be present in some fluids.

75 (684)

Observations on the relation of carbon dioxide and oxygen to the development of certain amphibian embryos.

By **A. M. BANTA.**

[*From the Station for Experimental Evolution, Cold Spring Harbor, Long Island, New York.*]

The following observations were made during the past two seasons upon material kept for other purposes.[1]

Eggs of all the species mentioned below were placed when fresh or in early cleavage in artesian water containing 1.32 per cent. of CO_2, the amount normal to large open ponds here being only about 0.04 per cent.

Ambystoma punctatum.—Development was at the normal rate with no mortality of embryos traceable to the CO_2 in the water. The larvæ likewise lived fairly well, though in many cases not so well in the CO_2 water as in pond water.

Spelerpes bilineatus.—Cleavage and later development were probably at the normal rate, but there was a large mortality percentage in standing or running artesian water. The mortality was less in standing water from ponds but thoroughly oxygenated water comparatively free from CO_2 was necessary to get the highest percentage of developing embryos.

[1] Observations on *Spelerpes*, *Rana pipiens* (?), and *Rana sylvatica* were made on material kept in collaboration with Dr. R. A. Gortner, of this station.

Bufo lentiginosus.—Only a small percentage developed at all and none beyond early cleavage.

Hyla versicolor and *Hyla pickeringii*.—A few went through cleavage but none beyond.

Rana pipiens (?).—Mortality was very large during early stages but perhaps 5 per cent. developed until the embryos were considerably differentiated and about 3 mm. in length. None developed further. In two of the ponds observed all the eggs and embryos of this species died much as those placed in CO_2 water in the laboratory. These were ponds practically without aquatic plants and containing great quantities of decaying leaves and other plant debris and therefore doubtless had much CO_2 in the water.

Rana sylvatica.—The development was apparently at the normal rate. In many cases the larvæ hatched, but though active for a day or two and clinging to the jelly mass and sides of the jars in the usual fashion took no food and developed no further. All died within a few days. In other jars where considerable masses of the eggs were placed and where large numbers died before hatching the larvæ in the interior of the masses and thus least exposed to the CO_2 water survived longest.

Often the eggs of the inner portions of the egg masses of *Rana sylvatica* and *Ambystoma tigrinum* (all of which were otherwise developing normally) show a retarded development, progressively the more so the farther the eggs are from the periphery of the mass, due to insufficient oxygen. In one egg mass of *Ambystoma* the exterior embryos of the mass were 10.2 to 11 mm. long and almost ready to hatch, while at the other extreme those deepest within the mass were only 3.6 mm. long and still in the late neural groove stage. In most cases in the ponds where the *Ambystoma* eggs are laid, as well as in jars in the laboratory, a portion of the interior of the masses die before hatching. In one pond near the laboratory protected from wind and containing very quiet and poorly oxygenated water perhaps 90 to 95 per cent. of the eggs die each year. That insufficient oxygen is the cause was indicated by the low percentage mortality in a few masses left almost entirely exposed to the air by the lowering of the level of the pond. These conditions were imitated in the laboratory with the result

that in some cases 60 to 100 per cent. of the eggs of egg masses kept in a depth of 12 to 18 inches of quiet, poorly oxygenated water died, while in similar adjoining vessels eggs less than half submerged developed almost without exception.

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Direct observation of cell division in mammalian tissue.

By **ROBERT A. LAMBERT.**

[*From the Department of Pathology, College of Physicians and Surgeons, Columbia University.*]

Connective tissue cells of the rat cultivated outside the body in rat plasma for as long as seventy-six days formed the material for observation. For maintaining activity for such a period, the pieces of tissue were transferred to fresh plasma at intervals of five to ten days. Measures employed by Carrel for rejuvenating the cells in old cultures were found to be unnecessary.

We have found that the process of karyokinetic division may be followed in the living cells. The earlier phases often escape notice. The later phases, however, may be observed with ease even with the lower powers of the microscope. In actively growing cultures the time required for the entire process is from forty to sixty minutes. The time elapsing between the divergence of the masses of daughter chromosomes to complete division of the cytoplasm averages about ten minutes. In slowly growing cultures the process may be retarded. A half hour to an hour after division is required for the daughter cells to develop the form and staining qualities of the resting cell. These time periods relate to observations made on cells at 35–37°C. At lower temperatures (25–30°) the process is slower.

Cells containing numerous fat droplets have been seen to divide as rapidly as cells free from fat.

Amitotic division has not been observed.

Cells after division have been followed and further division noted. The size reached by these daughter cells was approximately that of the parent cells. This observation affords proof that true growth takes place in cells cultivated in vitro.

77 (686)

The effect of drying upon the viability of bacteria.

By **C. E. A. WINSLOW** and **F. ABRAMSON.**

[*College of the City of New York.*]

The curve of viability, measuring the rate of decrease among bacteria exposed to an unfavorable environment, has been determined for many conditions. Drying is known to cause a rapid elimination, but no exact quantitative determinations have been made. In the experiments here reported, colon bacilli were used. One cubic centimeter of a 48-hour broth culture was mixed with 2½ grams of sea sand spread out in an ordinary Petri dish. Duplicate dishes prepared in this way were covered with porous earthenware tops and kept for various periods of time from four hours to ten days. At the end of each period nine cubic centimeters of sterile water were added to the sand in one of the dishes and well mixed by careful agitation. Portions of this water were then plated in duplicate on agar in the usual manner.

The results of eight series of tests are shown in the table below expressed in percentages of the original number of bacteria present. The original numbers in different series varied from 4,150,000 to 117,300,000 per gram of sand, and the final number remaining after ten days in Series II and III were 20,700 and 18,900 per gram, respectively. The average temperature and humidity of the room in which the plates were kept is shown for the last five series at the bottom of the table.

PERCENTAGE OF ORIGINAL NUMBER SURVIVING.

Period. Hours.	Series.							
	I.	II.	III.	IV.	V.	VI.	VII.	VIII.
4						76.45		
5½						57.18		
6				75.90			79.82	
7					57.56	37.46		57.76
8				67.23	59.30		69.03	
10					44.48			41.38
24	.85	.85	.85					
48	.16	.16	.15					
120	.08	.06	.06					
166	.04	.04	.04					
216		.02	.02					
240		.02	.02					
Average temperature.....				69°	69°	70°	69°	69°
Average relative humidity ...				90%	72%	60%	90%	72%

The results are quite concordant when the dryness of the atmosphere is taken into consideration. Series VI showed a more rapid reduction than the others during the first 7 hours and the relative humidity was 60 per cent. Then came Series V and VIII with a relative humidity of 72 per cent., while in Series IV and VII with an atmospheric humidity of 90 per cent., nearly 70 per cent. of the bacteria were alive after 8 hours.

The general rate of reduction was directly proportional to the time, during the first 24 hours, and then fell off more and more gradually. As a rule, intestinal bacteria in any foreign medium, colon bacilli in water, for example, decrease rapidly at first and more and more slowly as time goes on, following almost a parabolic curve. Most die at first, but a few persist for a long time. In its general relations the curve for drying is similar. 99 per cent. of the bacteria are gone after 24 hours while 2 out of 10,000 persist after 10 days. The curve for the first 24 hours is however here practically a straight line. 50 per cent. perished after 10 hours, and 99 per cent. after 24 hours. This is to be explained by the fact that during this period the bacteria were not exposed to constant conditions, since the originally moist sand was becoming progressively dryer. Determinations made by weighing at intervals duplicate samples prepared just like those which were inoculated with the bacteria showed that nine tenths of the moisture in the sand was gone after 10 hours and practically all of it was gone after 24 hours (with an atmospheric humidity of 80 per cent.). With a constant unfavorable environment, the reduction of bacteria proceeds at a decreasing rate. With an environment growing more and more unfavorable we might expect an approximately even rate of reduction such as is indicated here.

So far as the absolute reduction is concerned, it appears that drying is highly inimical to the bacteria studied. A uniform reduction of 99 per cent. is indicated after 24 hours. It takes over a week to reach such a point when colon bacilli and similar intestinal forms are stored in water.

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Determination of the amino-acid nitrogen in the urine.

By **S. R. BENEDICT** and **J. R. MURLIN.**

[*From the Cornell University Medical College.*]

Since the abandonment of the Pfaundler method for determination of the amino-acid nitrogen of the urine, the only direct methods proposed which have met with any favor, are the gasometric method of D. D. Van Slyke and the titration method of Henriques and Sörensen. In our hands the Van Slyke method as originally described[1] has not proved entirely satisfactory, (1) because of the difficulty of removing all the ammonia after conversion of urea, and (2) because poly-peptids and other condensation products of amino-acids, *e. g.*, hippuric acid, are estimated as well as free amino-acid nitrogen. The method, therefore, probably gives results which are too high.

The Henriques and Sörensen method as improved by the authors themselves[2] likewise presents some difficulties. For example, as objected by de Jager and since admitted by Henriques and Sörensen, in the presence of large quantities of ammonia the total titration is less than the sum of the ammonia N and amino-acid N done separately. It is therefore necessary first to remove the ammonia. Henriques and Sörensen recommend for this the method of Krieger and Reich slightly modified. This is essentially the method well known in this country by Shaffer's name. Since the method is based on distillation under diminished pressure, it is not adapted to rapid determinations in a series of urines simultaneously. Besides, as used by Henriques and Sörensen, we have not been able to obtain as high ammonia figures as by the Folin method. Use of the latter method for removal of ammonia, while perfectly satisfactory for small samples of urine (10 or 20 c.c.) is not satisfactory for a sample large enough to give a titration for amino-acid nitrogen (40–50 c.c.).

Another objection to the Henriques and Sörensen procedure is the difficulty of titration with phenolphthalein in a barium filtrate because of interference of carbon dioxide.

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[1] PROC. SOC. EXP. BIOL. AND MED., 1910, VII, p. 47.

[2] *Zeitschr. f. physiol. Chemie*, 1909, LXIV, p. 120.

These various difficulties have led us to return to the precipitation of ammonia by means of phosphotungstic acid. Gumlich[1] showed originally that the proper conditions for removal of ammonia by 10 per cent. phosphotongstic acid are a strongly acid urine and sedimentation for 24 hours. We have satisfied ourselves that this strength of phosphotungstic acid does not precipitate the mono-amino acids in the concentration usually found in urines. Instead, however, of removing the urea from the phosphotungstic filtrate and the determination of amino-acid nitrogen by difference, as in the original Schöndorff-Pfaundler method, or the modifications of it by Krieger and Schmidt, Van Leersum, and others, we remove the phosphotungstic acid by means of tribasic lead acetate and litharge, and titrate for mono-amino acids in the filtrate (after removal of lead) according to the procedure of Henriques and Sörensen. The filtrate is water-clear and free of any constituents which can interfere with the formalin titration.

COMPARISON OF THE HENRIQUES AND SÖRENSEN METHOD WITH MODIFIED METHOD.

Urine	No.	H. and S.	Modified Method.
Case 1. Pernicious vomiting.	Urine No. 1	0.215 gm. NH_2N	0.015 gm. NH_2N
	Urine No. 2	0.434 gm. NH_2N	0.048 gm. NH_2N
	Urine No. 4	0.268 gm. NH_2N	0.025 gm. NH_2N
	Urine No. –	0.245 gm. NH_2N	0.023 gm. NH_2N
Case 2. Eclampsia.	Urine No. 3	0.397 gm. NH_2N	0.120 gm. NH_2N
	Urine No. 7	0.157 gm. NH_2N	0.035 gm. NH_2N
	Urine No. 8	0.375 gm. NH_2N	0.148 gm. NH_2N
	Urine No. 11	0.210 gm. NH_2N	0.155 gm. NH_2N

All the urines contained a large amount of ammonia. It would seem from this comparison that the Henriques and Sörensen method like the Van Slyke method gives results (especially on pathological urines) even after the supposed removal of ammonia which may be quite misleading.

The following are a few of the results obtained with pure substances.

A. Pure Solutions:

1. 20 c.c. *N*/20 leucine, purity tested by Kjeldahl and formol titration, added to 5 c.c. *N*/10 NH_4Cl < 200 c.c.; 45 c.c. of final filtrate, titration 2.4, theory 2.28.

[1] *Zeitschr. f. physiol. Chemie*, 1893, XVII, p. 13.

2. 20 c.c. leucine + NH_4Cl < 180 c.c.; 50 c.c. final filtrate, titration 2.7, theory 2.77.
3. Mixture of urea, $(NH_4)_2SO_4$, uric acid, alanin, glycocoll, glutamic acid (all tested substances), containing 0.144 gm. amino-acid nitrogen, yielded by new method 0.132, 0.148, 0.143 gm.

B. Pure Substances added to urines.

1. 200 c.c. normal urine; 100 c.c. final filtrate, titration 9.6 c.c. *N*/10 NaOH.
   200 c.c. normal urine + 20 c.c. *N*/20 leucin; 100 c.c. filtrate, titration 11.8 NaOH
   Difference 2.2 c.c., theory 2.5 c.c.
2. 200 c.c. urine of puerperient woman; 100 c.c. filtrate, titration 9.7 *N*/10 NaOH.
   200 c.c. urine of puerperient woman + 20 c.c. *N*/10 alanin, titration 14.7 *N*/10 NaOH.
   Difference 5.0 c.c. *N*/10 NaOH, theory 5.0 c.c.

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Picrolonates of the monoamino acids.[1]

By **P. A. LEVENE** and **DONALD D. VAN SLYKE.**

[*From the Rockefeller Institute for Medical Research, New York.*]

Picrolonic acid, used by Steudel to precipitate the hexone bases, and later shown by Mayeda to form salts with the aromatic amino acids tryptophane and phenylalanine, also forms crystalline salts of normal composition with the other monoamino acids obtained on hydrolysis of proteins. The salts are made by dissolving molecular proportions of amino acid and picrolonic acid in a minimum amount of boiling water. The picrolonates crystallize from the cooling solutions, usually while they are still warm. In cold water many of them are very insoluble. In alcohol they are all more soluble than in water. Following is a list of the amino acids of

[1] After this title had been sent to the secretary an article by Abderhalden and Weil appeared describing picrolonates of glycocoll; d-alanine, and dl-leucine (*Ztschr. physiol. Chem.*, 78, 150). They were formed in alcoholic solution, which yielded products of abnormal composition in the cases of glycocoll and alanine.

which picrolonates were prepared, after each being given the solubility of the picrolonate in grams per 100 c.c. of water at 20°–23°, and the melting point. The picrolonates are arranged in order of solubility. *Dl-phenyl-alanine*, 0.12, 212° (decomp.); *Tyrosine*, 0.29, blackens at 260°; *l-phenyl-alanine*, 0.34, 208°; *dl-leucine*, 0.53, indefinite above 140°; *l-leucine*, 0.55, indefinite at about 150°; *d-isoleucine*, 0.58, 170°; *dl-valine*, 0.81, indefinite above 150°; *dl-serine*, 0.98, decomposed 265°; *glycocoll*, 0.99, 214°; *dl-alanine*, 1.01, 216°; *d-valine*, 1.20, 180°; *d-alanine*, 1.61, 214°; *dl-aspartic* acid, 1.69, blackens at 130°; *dl-glutaminic acid*, 2.37, 194°.

Nearly all the picrolonates decompose more or less on melting, and the melting points are not sharp. L-Phenyl-alanine picrolonate is much more soluble in alcohol than the racemic salt, and can be separated from most of the racemic substance by solution in alcohol. The optically active compound has a sp. rotation in absolute alcohol of + 30°. The specific rotation of d-isoleucine picrolonate in alcohol is + 33.3°. Phenyl-alanine can be separated from glutaminic or aspartic acid by dissolving the mixture with enough picrolonic acid to combine with the phenyl-alanine only. The picrolonate of the latter crystallizes pure. It is probable that other amino acids can be separated by similar use of picrolonic acid. The pyrrollidine acids, proline and oxyproline, do not readily yield picrolonates when treated as described above.

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An improved apparatus for gasometric determination of amino nitrogen.

By **DONALD D. VAN SLYKE.**

[*From the Rockefeller Institute for Medical Research, New York.*]

The apparatus differs from that reported at the meeting of this society in December, 1909, in that: (1) The desamidizing bottle with stopper holding the 10 c.c. burette and tubes is replaced by a bulb into which the tubes and burette are sealed; (2) the Hempel absorption pipette is modified so that it can be suspended from hooks; (3) both absorption pipette and desamidizing bulb are so arranged that they can be shaken by a motor. The apparatus,

without losing any of the accuracy of the original, is much more convenient because it can be used an indefinite number of times without disconnecting the parts, and the shaking by hand is replaced by the less laborious and more efficient motor. The latter also increases the rapidity, so that with one apparatus a determination can be made every seven or eight minutes.

81 (690)

The nature of the free amino groups in the native proteins.

By **DONALD D. VAN SLYKE** and **F. J. BIRCHARD.**

[*From the Rockefeller Institute for Medical Research, New York.*]

The fact that at least some proteins contain a small but definite proportion of their nitrogen in the form of free amino groups capable of reacting with nitrous acid has been proven by one of us. Levites and Skraup failed to obtain lysine from proteins which had been treated with nitrous acid before hydrolysis. This indicates that the ω-NH_2 group of lysine, $\mathbf{NH_2}\cdot(CH_2)_4\cdot CH_2(NH_2)$-COOH, may be free in the protein molecule. As lysine is peculiar among the amino acids in possessing an ω-NH_2 group, it appeared possible that this might be responsible for all or most of the amino nitrogen determined. We have, therefore, determined the free amino nitrogen in ten proteins in which the lysine has already been quantitatively determined in either our laboratory or in Osborne's.

Protein.	Per Cent. of Total N in Lysine.	½ Lysine N.	Free Amino N.
Ox-hemoglobin	12.0 (Van Slyke)	6.0	5.6
Fibrin	11.4 (Van Slyke)	5.7	5.3
Hemocyanin	8.5 (Van Slyke)	4.25	4.3
Casein	6.9 (Osborne)	3.45	3.4
Gelatin	6.3 (Van Slyke)	3.15	3.1
Egg albumin	4.0 (Osborne)	2.0	2.4
Edestin	3.8 (Van Slyke)	1.9	1.2
Zein	0.0 (Osborne)	0.0	0.3
Albumoses from Witte Pepton.			
Heteroalbumose	10.3	5.15	6.3
Protoalbumose	9.6	4.8	6.6

Without exception the free amino N of the native proteins

equals within a fraction of a per cent. that of the ω-group of the lysine. It appears that the ω-group of lysine constitutes practically all of the free amino nitrogen of the native proteins determinable with nitrous acid. The albumoses show appreciably more free amino nitrogen, which is to be expected from the fact that the protein molecule is partially broken down in their preparation.

82 (691)

An experimental study of anti-anaphylaxis.

By **R. WEIL** and **A. F. COCA.**

[*From the Laboratories of Experimental Therapeutics and Experimental Pathology, Cornell University Medical School, New York City.*]

If a guinea-pig be given a single injection of a foreign proteid, it becomes, after the lapse of 10 to 14 days, actively sensitized to that proteid, in such wise that the reinjection of the same, in doses far too small to cause any symptoms in a normal animal, produces almost immediate death with convulsions. If, however, such a sensitized pig, after the sensitizing injection, be given a second dose, too small to induce its death, it immediately passes into a condition of anti-anaphylaxis, in which it is refractory to the foreign proteid in question, and may manifest no symptoms even after the injection of doses toxic to normal animals. This refractory stage lasts for weeks or months. In the same way, an animal may be passively sensitized by the introduction into its veins or peritoneum of the serum of another animal, which has been previously immunized or sensitized to a foreign proteid; in this case, too, the injection of a relatively small, *i. e.*, sublethal, dose of the same proteid into the passively sensitized animal produces a condition of anti-anaphylaxis.

By no experimental device hitherto employed has it been possible to alter this condition of anti-anaphylaxis. The theories offered to explain it are numerous. Friedemann, in his general review of anaphylaxis, in 1911, cites three hypotheses, those of Gay and Southard, of Besredka, and of Friedberger, all of which he proves to be untenable, and offers three other possible explana-

tions. He concludes that anti-anaphylaxis presents a "most interesting, novel, and as yet unexplained phenomenon."

The following experiments appear to us to throw much light on the problem. Guinea-pigs have been actively sensitized, by injection, to a foreign proteid; after the lapse of about two weeks, they have been rendered anti-anaphylactic by the intra-peritoneal injection of a sublethal dose of this proteid. (That anti-anaphylaxis had actually been so induced was demonstrated by the injection of relatively enormous doses of the proteid into controls similarly treated, without toxic effect.) The anti-anaphylactic animals have then been *re-sensitized.* This re-sensitization is accomplished by bleeding to death another guinea-pig, sensitized to the identical proteid, and then injecting his serum, in amounts of from 2 to 5½ c.c., into the veins of the anti-anaphylactic animal. After the lapse of less than 24 hours, the re-sensitized pig has been tested by an intravenous injection of the foreign proteid. In each and every case, the pig, upon undergoing this test, has manifested the typical symptoms of anaphylaxis, with immediate death. In other words, such a pig acts exactly like a normal pig, which has been passively sensitized by the introduction of serum from an anaphylactic pig.

If instead of a sensitized pig, one makes use of the serum from a pig highly immunized by means of repeated injections, re-sensitization of the anti-anaphylactic animal is accomplished in the same manner.

If an actively anti-anaphylactic pig be passively re-sensitized with the serum of a rabbit immunized to the same foreign proteid, he too becomes hypersensitive, and is killed by a subsequent dose of this proteid.

The same experiments can be performed in the case of animals rendered anti-anaphylactic after passive sensitization. If, during the refractory period, say two days after the anti-anaphylactizing dose, they are re-sensitized to the same foreign proteid by the reintroduction of an immunized rabbit's serum, they may be killed in the typical manner by the re-injection of the proteid. The only difference between passive anaphylaxis as primarily and as secondarily induced, is that in the latter somewhat larger doses are required to re-sensitize. The cause of this difference is being investigated and will form the subject of a later report.

These experiments in anti-anaphylaxis were originally performed with horse serum. In order to avoid any possible source of error, however, due to the complex character of this material they were repeated with a solution of crystalline egg albumen which had been four times re-crystallized, and the same results were obtained.

It appears from these experiments that anti-anaphylaxis is a condition in which an animal becomes refractory to the toxic effects of a foreign proteid simply through the exhaustion from his blood of those bodies which induce the reaction. The proof hereof lies in the fact that the simple re-introduction of these bodies with the blood of another sensitized animal restores him at once to his original condition of anaphylaxis. The bodies which induce the reaction are, so far as we know, two: first, the "anaphylactic anti-bodies," which resemble in character, and may be identical with, the amboceptors; second, the complement substances of the blood.

It is unlikely that the complement is the substance herein at fault, inasmuch as Friedemann states, as the complement is rapidly restored to its normal amount in anti-anaphylactic pigs, whereas the condition of anti-anaphylaxis persists. We have tested whether the complement plays an important rôle by two sets of experiments. In the first place, the animals have been simultaneously sensitized by hypodermic injection to two different forms of foreign proteid, namely horse serum and egg albumen. They have then been rendered anti-anaphylactic to one of these. Immediately, thereafter, it has been found that their sensitiveness to the other albumen is little, if at all, impaired. This indicates that the complement cannot be deficient. In the second type of experiments, the pigs, having been made anti-anaphylactic to egg albumen, have been given an intra-venous injection of 5 c.c. of normal guinea-pig serum, which would, of course, suffice to supply any defect of complement. When now re-injected with egg albumen, they fail to evidence any sensitiveness. This indicates, therefore, that something other than complement, necessary to the anaphylactic reaction, has been removed from the blood in anti-anaphylaxis. It seems to us, therefore, that animals are anti-anaphylactic simply through the absence of the appropriate

anti-bodies from the serum. If these are supplied, an animal passes directly into the anaphylactic condition again, and this reversal could conceivably be repeatedly renewed.

Conclusions.

1. Guinea-pigs which have been rendered actively anaphylactic by a preliminary injection of foreign proteid (horse serum, egg albumen), and have then been made anti-anaphylactic by the injection of a sub-lethal dose of this proteid, may be immediately re-sensitized by the introduction of serum from a pig sensitized, or one immunized to the same proteid. Death is then produced, with typical anaphylactic symptoms by the intravenous injection of an amount of this proteid which to normal pigs is non-toxic.

2. Actively anti-anaphylactic pigs may be re-sentitized with serum derived from a rabbit immunized against the same proteid.

3. Passively sensitized pigs, rendered anti-anaphylactic by the usual methods, can be re-sensitized by the re-introduction of an immune serum.

4. A pig simultaneously sensitized to two different foreign proteids, may be rendered anti-anaphylactic to one of these by the usual methods, while its sensitiveness to the other remains unimpaired. This demonstrates that anti-anaphylaxis cannot be due to exhaustion of complement, since complement is amply present.

5. The introduction of normal guinea-pig's serum into an anti-anaphylactic animal fails to re-sensitize. Some factor other than complement, therefore, must be introduced in re-sensitization.

6. The absence of available anaphylactic antibodies appears to be the cause of the refractory condition known as anti-anaphylaxis, and re-sensitization is due to their re-introduction.

83 (692)

The toxicity of foreign leucocytes.

By **Wilfred H. Manwaring.**

[*From the Rockefeller Institute for Medical Research.*]

The injection of from 0.7 c.c. to 1.0 c.c. of rabbit leucocytes into the cerebral meninges of dogs is apparently invariably fatal.

The animals show no immediate symptoms; but about two hours later there is beginning incoördination, rigidity and respiratory distress, increasing to collapse about the fourth hour, and death about the sixth. An occasional dog survives till the twenty-fourth hour. Autopsy usually shows extensive local hemorrhagic, inflammatory and necrotic changes.

Horse leucocytes similarly injected produce symptoms of the same general nature, though less severe. About two thirds of the dogs injected with horse leucocytes recover. A reinjection of these dogs, however, or the injection of dogs whose meninges have been previously injured by tubercle bacilli, is almost invariably fatal.

The injection of 0.5 c.c. of rabbit leucocytes into the spinal meninges of monkeys produces slight symptoms from which most of the monkeys recover. Larger amounts are usually fatal.

Horse leucocytes are less toxic for monkeys, producing few if any symptoms, even when injected in 1.0 c.c. doses. The toxicity of both leucocytes however increases on repeated injection, the third injection often being fatal. Autopsy in such cases often shows edema of the lungs as the apparent immediate cause of death.

These tests have a bearing on the possible therapeutic uses of leucocytes in meningeal infections.

84 (693)

The occurrence, and the significance, of tyrosinase in the reproductive organs of certain amphibians.

By **ROSS AIKEN GORTNER.**

[*From the Laboratory of Biological Chemistry of the Station for Experimental Evolution, The Carnegie Institution of Washington.*]

Tyrosinase—the enzyme which oxidizes tyrosin to produce a black, insoluble, pigment-like compound—has been shown by Phisalix (*C. R. Soc. Biol.*, 50, p. 793, 1898) to occur in the skin of the European frog, *Rana esculenta*. Gessard, later (ibid., 56, p. 285, 1904), shows that the same enzyme occurs in the skin of the toad, *Bufo vulgaris*, and in the frog, *Rana temporaria*. In discussing the

importance of the tyrosinase Phisalix says: "It is probable that the oxidase presides over the oxidations within the organism . . . and plays the rôle of fixing oxygen in cutaneous respiration." If such is the case, and the production of the pigment in the skin is only a secondary reaction, or the elimination of a byproduct, the study of this enzyme becomes of much greater importance than merely as a pigment-producer.

In every instance where the mechanism of melanin formation has been elucidated, it has been found that pigmentation is due to the interaction of tyrosinase and a chromogen. It therefore seemed probable that the enzyme might be present in the ovaries of those amphibians which deposit pigmented eggs, and might be responsible for the pigment formation. I have found that tyrosinase is present in the ovaries of the green frog, *Rana clamata*, and the wood frog, *Rana sylvatica*. Owing to the greater ease in securing material of various ages most of the tests were carried out using material from the green frog. *In every instance the intensity, and the rapidity of the development of the tyrosinase test was in inverse proportion to the amount of pigment present in the eggs*. Ovaries which were immature, and contained no pigmented eggs, gave a positive tyrosinase test in a very few hours, partially pigmented ovaries required in some instances as much as 96 hours to produce a positive test, while the ovaries which contained ripe eggs failed to give any indication of the presence of the enzyme in 168 hours. All solutions were kept sterile by the addition of a few drops of chloroform. When partially pigmented ovaries were used the "blank" (no tyrosin added) usually showed considerable darkening, which, however, did not appear in a boiled check, showing that a chromogen was also present. It would, therefore, appear that the pigmentation of the eggs of the frog is due to an oxidation induced by tyrosinase, and that as the pigmentation progresses the amount of tyrosinase decreases, until there is no perceptible amount of the enzyme present in the unfertilized eggs which are fully ripe.

The tadpoles at the moment of liberation from the egg contain tyrosinase, as do also the larvæ of the salamander, *Ambystoma punctatum*. Inasmuch as the ripe unfertilized ova do not seem to contain tyrosinase, it seemed probable that the enzyme might be

added through the sperm, and on testing the testes of adult green frogs during the breeding season, I found tyrosinase to be present, although the coloration was slow to develop (72 hrs.). It is possible that all of the tyrosinase in the ovary was used up in the production of the egg pigment, and that the oxidase for the tadpole is introduced by the male. It is well known that oxidative processes proceed much more rapidly after fertilization and perhaps we may find that in other instances this is due to the entrance of an oxidase with the sperm. Evidences of oxidase action have been found in all of the fertilized amphibian eggs that I have examined, including eggs which contain no pigment.[1] It is also possible that the "poisonous complex," to which Loeb (*Arch. Entwick. Organ.*, 31, p. 658) ascribes the death of the unfertilized egg, is destroyed by the entrance of an oxidase (perhaps a *specific* oxidase) with the sperm.

85 (694)

On two different types of melanin.

By **ROSS AIKEN GORTNER.**

[*From the Laboratory of Biological Chemistry of the Station for Experimental Evolution, The Carnegie Institution of Washington.*]

In investigating the nature of the melanin molecule, I have found that the pigment which is present in black wool is readily soluble in dilute sodium hydroxide, and that it is apparently a protein. To pigments of this nature I have given the name of melano-protein to distinguish them from both the unpigmented proteins and those other melanins, the nature of whose molecule is as yet unknown. The melano-protein which I have obtained from black wool contains no ash, showing that ash is not a part of this pigment, and also proving that this melanin does not contain iron. In some of the preparations of pigment from black wool where less precautions were taken to insure the absence of all contaminating mineral matter, a low percentage of ash was ob-

[1] In collaboration with Dr. Banta, of this station, I have recently had occasion to test fertilized eggs of *Rana sylvatica, Rana pipiens, Ambystoma punctatum,* and *Spelerpes bilineatus.*

tained (0.10 per cent.–0.20 per cent.) but this ash appeared as white particles and was probably silica.

When I undertook to prepare a melanin from black rabbit hair and black feathers I found that the pigment was very *insoluble* in dilute (0.2 per cent.) sodium hydrate, and it was only after long boiling, in some instances nearly a week, that solution was effected. Of course this procedure altered the nature of the melanin molecule, but the fact that was of chief interest was that the resulting product contained between 2 per cent. and 3 per cent. of ash *and that this ash was chiefly iron oxide.* I have recently observed that there are probably at least two pigments in the darker colors of horse hair, one of these being a melano-protein with a very low ash content, and the other containing approximately 3 per cent. of ash *which is chiefly iron oxide.*

These pigments have been prepared in such a manner as to preclude any iron entering through contamination, and inasmuch as other pigments, *prepared by exactly the same process*, contain no iron, or at most only traces, we must conclude that *in some instances melanins may contain iron as a part of the molecule*, but that all melanins do not contain iron. Perhaps in this instance the oxidase acted on the hemoglobin, or some other iron complex, instead of oxidizing a protein containing no iron.

86 (695)

A five-year pedigreed race of Paramæcium without conjugation.

By **LORANDE LOSS WOODRUFF.**

[*From the Sheffield Biological Laboratory, Yale University.*]

The unicellular organisms afford a natural means of approach to the problem of fertilization, and the study of data, from a long series of careful experimental studies on these forms by various investigators, has pointed to the conclusion that the most important function of conjugation in the life history of the Protozoa is a satisfying of an inherent periodic physiological need of living matter, resulting in a renewal of the vigor of the cell. This "dynamic" view of fertilization has gradually assumed a com-

manding position, though it is neither contradictory nor confirmatory of the view that fertilization, resulting in amphimixis, is concerned in some way with the phenomenon of variation, or that it may be a process which enables certain forms to withstand changed environmental conditions.

The present paper briefly outlines the results which have been obtained to date from an intensive study of a pedigreed race of *Paramœcium aurelia* with reference to the problem of protoplasmic senescence and the function of conjugation. I have previously published[1] the results obtained to September, 1910, and the reader is referred to earlier papers for further details of the culture and for a general discussion of the various phases of the work.

This culture was started on May 1, 1907, with a "wild" *Paramœcium aurelia* which was isolated from a laboratory aquarium. This individual was placed in about five drops of culture medium on a glass slide having a central ground concavity, and when the animal by division had formed four individuals, each of these was isolated on a separate slide to form the four lines, Ia, Ib, Ic, and Id, of this culture, *Paramœcium* I. The pedigreed culture has been maintained by a specimen isolated from each of these lines practically every day up to the present time, thus precluding the possibility of conjugation occurring and facilitating an accurate record of the number of generations attained. A culture medium consisting of infusions of hay and fresh grass was employed during the first nine months of the work, but thereafter infusions of nearly any materials which might be found in ponds, swamps, etc., have been used. The medium has invariably been boiled to render the introduction of "wild" individuals into the culture absolutely impossible.

This race of *Paramœcium* has attained so far, (May 1, 1912) 3,029 generations during the five years it has been under daily observation. The number of generations attained during each of the first five years of its existence is as follows: first year 452, second year 690, third year 613, fourth year 612, and fifth year 662. The mean rate of division for the entire period is over three divisions in forty-eight hours. Periods of marked physiological depression have not occurred—such variations in the rate of

[1] *Archiv für Protistenkunde*, Bd. 21, 4.

reproduction as have appeared being either normal rhythms or the effects of environmental changes of temperature and culture medium. The organisms of the present generation are in as normal morphological and physiological condition as the original "wild" individual isolated to initiate the culture.

This study has demonstrated that, under favorable environmental conditions, the protoplasm of the cell originally isolated possessed (at least) the potentiality to produce similar cells to the number represented by 2 raised to the 3,029th power, or a volume of protoplasm approximately equal to 10^{1000} times the volume of the Earth. I believe this result proves beyond question that the protoplasm of a single cell may be self-sufficient to reproduce itself indefinitely, under favorable environmental conditions, without recourse to conjugation and clearly indicates that senescence and the need of fertilization are not primary attributes of living matter.

87 (696)

The influence of tartrates upon phlorhizin diabetes.

By **FRANK P. UNDERHILL.**

[*From the Sheffield Laboratory of Physiological Chemistry, Yale University, New Haven, Conn.*]

A recent communication of Baer and Blum (*Archiv für Experimentelle Pathologie und Pharmakologie*, 1911, 65, p. 1) shows that the subcutaneous administration of a number of organic compounds, containing two carboxyl groups, exercises a remarkable inhibitory influence upon the elimination of urinary nitrogen and dextrose in dogs with phlorhizin diabetes. Among the substances possessing this property may be mentioned glutaric and tartaric acids.

In an endeavor to explain the mechanism of the unique influence exerted by these compounds investigations have been carried out with tartrates upon both dogs and rabbits under conditions similar to those established by Baer and Blum. We have been able to corroborate the findings of Baer and Blum with respect to the action of tartrates although Ringer (PROC. SOC. EXP. BIOL.

AND MED., 1912, 9, p. 54) failed to obtain the reported results with glutaric acid.

Our interpretation of the diminution of the urinary constituents is, however, entirely different from that offered by Baer and Blum. Tartrates subcutaneously injected cause a prompt disintegration of the cellular elements of the kidney tubules, leading to partial or complete loss of secretory activity, and in many cases to anuria. Hence, in phlorhizin diabetes urinary nitrogen and sugar are not eliminated to an appreciable extent.

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EXECUTIVE PROCEEDINGS.

Forty fifth meeting.

Columbia University, October 18, 1911. President Morgan in the chair.

Members present: H. M. Adler, I. Adler, Alsberg, Auer, Bancroft, Calkins, Field, Jackson, Joseph, Levene, Levin, Meltzer, Morgan, Pappenheimer, Senior, Simpson, Stewart, Wallace, H. B. Williams.

Forty sixth meeting.

The Rockefeller Institute for Medical Research, December 20, 1911. President Morgan in the chair.

Members present: Atkinson, Auer, Cohn, Davenport, Emerson, Eisenbrey, Field, Foster, Famulener, Fitzpatrick, Gies, Goldfarb, Hess, Jacobs, Janeway, Joseph, Lambert, Lee, Levin, Loeb, MacCallum, Manwaring, Meltzer, Morgan, Mosenthal, Norris, Ottenberg, Pappenheimer, Rous, Steinhardt, Swift, Terry, Wallace, Weil.

Members elected: A. M. Banta, E. C. Dickson, E. M. Ewing, J. G. Fitzgerald, R. A. Gortner, A. E. Guenther, W. de B. MacNider, B. S. Oppenheimer, F. H. Pike.

Forty seventh meeting.

College of the City of New York, February 21, 1912. President Morgan in the chair.

Members present: Alsberg, Atkinson, Bancroft, Fitzpatrick, Githens, Goldfarb, Guenther, Janeway, Joseph, Karsner, Kast, Kleiner, Lambert, Lee, Longcope, Lusk, Mandel, Meltzer, Morgan, McCrudden, Parker, Pike, Ringer, Senior, Stewart, Storey, Terry, Wallace, Winslow.

Members elected: E. E. Butterfield, A. F. Coca, George Draper, G. Canby Robinson, H. Wastenays, C. J. Wiggers.

Officers elected: President, James Ewing; Vice-president, P. A. Levene: Treasurer, Charles Norris; Secretary, George B. Wallace.

Resolution adopted: Resolved, that groups of members be allowed by special vote of the society to form local branches of the society: that such local branches be designated by local names, *e. g.*, "The San Francisco Branch of the Society for Experimental Biology and Medicine;" and have power to hold meetings independently of the meetings of the society as a whole; that the expenses of such local meetings be borne by the local branches; that the members of such local branches have power to arrange their own programs and to publish their scientific papers in the proceedings of the society; and that only members of the society as a whole and who shall have been duly proposed to the council and elected in the manner provided for by the constitution be members of the local branches.

Forty eighth meeting.

Cornell University Medical College, April 17, 1912. President Ewing in the chair.

Members present: Auer, Bancroft, Benedict, Butterfield, Cohn, Ewing, Field, Fitzpatrick, Foster, Githens, Guenther, Hatcher, Kleiner, Lee, Levine, Longscope, Lusk, Manwaring, McCrudden, Meltzer, Mendel, Morgan, Murlin, Norris, Pappenheimer, Robinson, Steinhardt, Stewart, Stockard, Swift, Terry, Van Slyke, Wallace, Wiggers, H. B. Williams.

Members elected: J. H. Austin, H. E. Jordan, C. C. Lieb, F. W. Schlutz.

Resignation accepted: V. C. Vaughan.

Resolution adopted: Resolved, that abstracts of communications to be published in the Proceedings of the meeting at which they are presented must be delivered to the secretary not later than two days after the meeting; Otherwise they shall be published in the Proceedings of a subsequent meeting.

Forty ninth meeting.

University and Bellevue Hospital Medical College, May 15, 1912. President Ewing in the chair.

Members present: Auer, Bancroft, Banzhaf, Benedict, Birchard,

Butterfield, Calkins, Coca, J. W. Draper, Emerson, James Ewing, E. M. Ewing, Githens, Goldfarb, Harris, Hatcher, Jackson, Kleiner, Lambert, Lieb, J. A. Mandel, McCrudden, Murlin, Norris, Pike, Rous, Terry, Wallace, Weil, Wiggers, H. B. Williams, Winslow.

Members elected: M. T. Burrows, E. F. DuBois.

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OF THE

SCIENTIFIC PROCEEDINGS.

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